

Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis

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Endometriosis is a common gynecological disease associated with pelvic pain and subfertility. We conducted a genome-wide association study (GWAS) in 3,194 individuals with surgically confirmed endometriosis (cases) and 7,060 controls from Australia and the UK. Polygenic predictive modeling showed significantly increased genetic loading among 1,364 cases with moderate to severe endometriosis. The strongest association signal was on 7p15.2 (rs12700667) for 'all' endometriosis ($P = 2.6 \times 10^{-7}$, odds ratio (OR) = 1.22, 95% CI 1.13–1.32) and for moderate to severe disease ($P = 1.5 \times 10^{-9}$, OR = 1.38, 95% CI 1.24–1.53). We replicated rs12700667 in an independent cohort from the United States of 2,392 self-reported, surgically confirmed endometriosis cases and 2,271 controls ($P = 1.2 \times 10^{-3}$, OR = 1.17, 95% CI 1.06–1.28), resulting in a genome-wide significant P value of 1.4×10^{-9} (OR = 1.20, 95% CI 1.13–1.27) for 'all' endometriosis in our combined datasets of 5,586 cases and 9,331 controls. rs12700667 is located in an intergenic region upstream of the plausible candidate genes *NFE2L3* and *HOXA10*.

Main

Endometriosis (MIM131200) is a disease affecting 6–10% of women of reproductive age¹ with substantial annual health costs² and health burden for individuals^{3, 4}. Common symptoms include

chronic pelvic pain, severe dysmenorrhea (painful periods) and subfertility. The causes of endometriosis remain uncertain despite over 50 years of hypothesis-driven research. Disease severity is classified using the revised American Fertility Society (rAFS) system⁵, assigning affected individuals to one of four stages (stages I–IV, defined as minimal to severe disease) based on lesion size and associated pelvic adhesions. However, it remains unclear whether the disease progresses through these stages, and it has been suggested that small lesions (present in disease stages I and II) represent an epiphenomenon rather than a disease entity⁶. Endometriosis risk is influenced by genetic factors^{7, 8, 9, 10, 11, 12, 13, 14} and has an estimated heritability of around 51%.

We genotyped 3,194 unrelated cases with surgically confirmed endometriosis recruited by the International Endogene Consortium, IEC (QIMR, Australia dataset, $n = 2,270$; Oxford, UK dataset, $n = 924$)¹⁵, using the Illumina Human670Quad BeadArray (Online Methods). We assessed disease stage from surgical records using the rAFS classification system^{5, 15} and grouped the subjects into two phenotypes: stage A (stage I or II disease or some ovarian disease with a few adhesions; $n = 1,686$, 52.7%) or stage B (stage III or IV disease; $n = 1,364$, 42.7%), or unknown ($n = 144$, 4.6%) (Supplementary Table 1). Illumina Human610Quad control genotypes for QIMR cases were available for 1,870 individuals in an adolescent twin study^{16, 17}. For the Oxford cases, we obtained Illumina Human1M-Duo genotypes for 5,190 UK population controls from the Wellcome Trust Case Control Consortium (WTCCC2). Although endometriosis affects only women, the Australian and UK control sets included men to maximize the power of the association detection on the autosomal chromosomes (Online Methods). We detected no significant autosomal allele frequency differences between the male and female control samples (Supplementary Fig. 1), indicating that the association signals would not be influenced by a differing female to male ratio in the cases and controls.

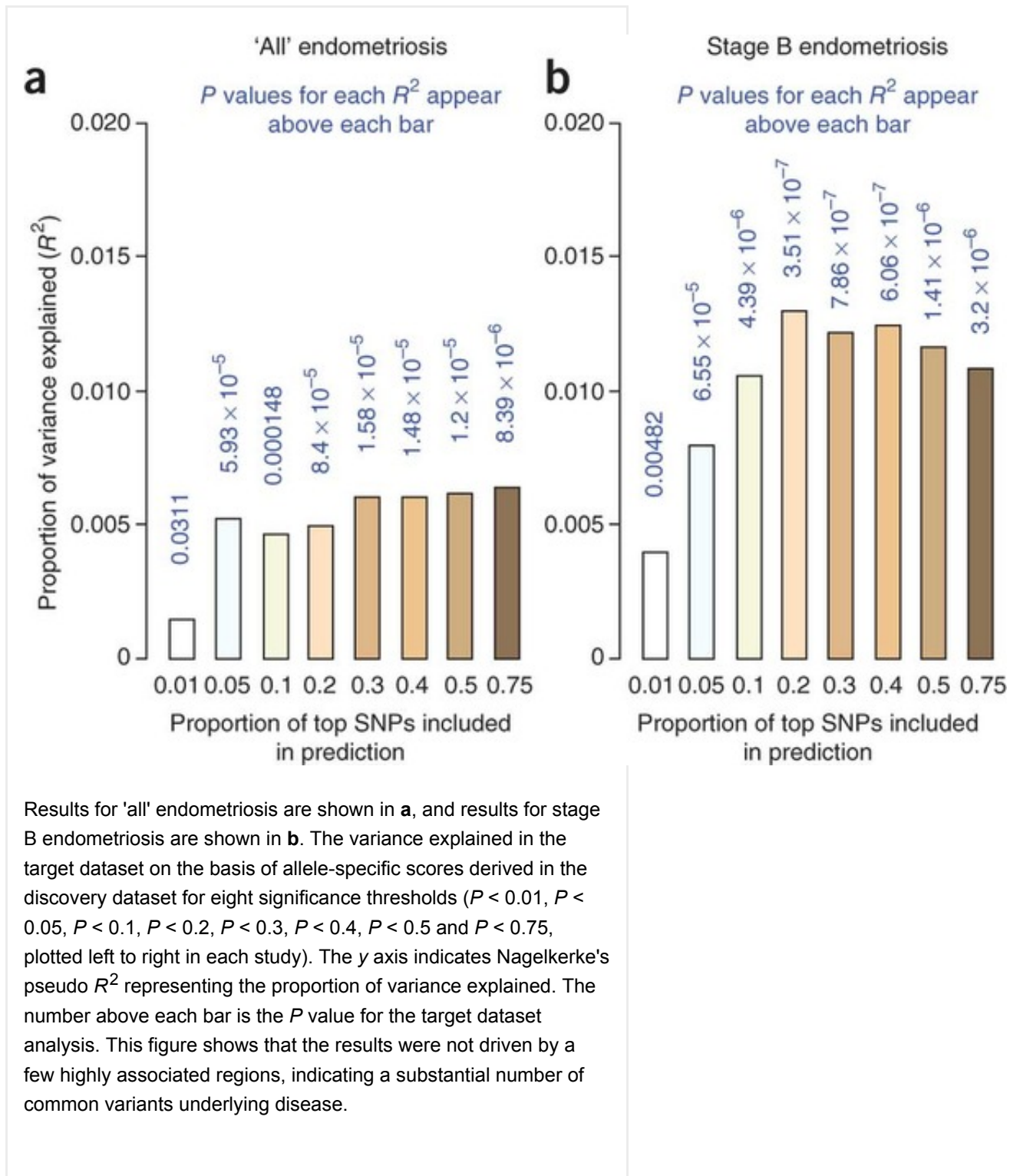
Studies to date have established that endometriosis is heritable but have not addressed the genetic burden for different disease stages. We used the GWAS data to assess genetic loading in cases in two complementary ways. Using a new method¹⁸, we estimated the proportion of variation in case-control status that can be explained by considering all SNPs simultaneously through inference of distant relatedness from marker data and comparing

it to case-control status (Online Methods). The proportion of variation in case-control status explained by the GWAS data was highly significant for both 'all' and stage B endometriosis (Table 1 and Supplementary Table 2). The estimate for stage B endometriosis (0.34, s.e. = 0.04) was significantly higher than that for stage A endometriosis (0.15, s.e. = 0.04; Table 1).

Table 1: Estimates of proportion of variation due to common genetic variants for 'all' endometriosis and stage A or B disease using genome-wide SNP data from cases and controls^a

We also assessed the genetic loading of the different stages using a prediction approach (Online Methods)¹⁹ in which we used the Oxford data as a discovery set to identify increasingly large SNP sets ranked on their significance of association ('allele specific scores') and used these scores to predict disease status in target samples from QIMR. The discovery and target sets were then reversed (Supplementary Fig. 2). Oxford 'all' endometriosis predicted endometriosis in the QIMR sample, with the smallest P value ($P = 8.4 \times 10^{-6}$) obtained for a score set including ~75% of the SNPs (Fig. 1). This result was highly significant, although the proportion of variance explained was small (maximum Nagelkerke r^2 of 0.007; 0.7% of the variance). For stage B cases, the proportion of variance explained by most score sets was higher; for example, the score set including the ~20% most associated SNPs ($P = 3.5 \times 10^{-7}$) explained 1.3% of the variance, consistent with a greater (polygenic) genetic loading for stage B disease.

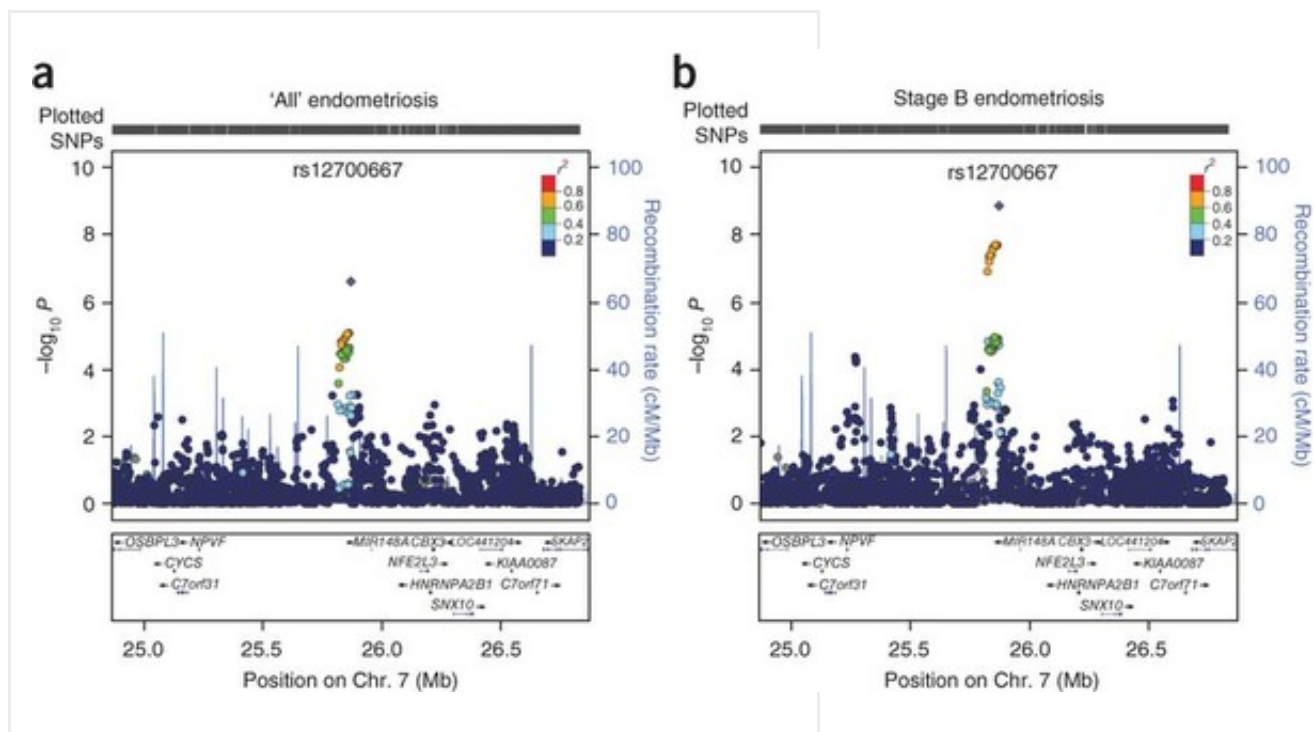
Figure 1: Allele-specific score prediction for endometriosis, using the Oxford population as the discovery dataset and the QIMR population as the target dataset.



We performed two genome-wide association analyses stratified by dataset (QIMR and Oxford) using (i) 3,194 'all' endometriosis cases and (ii) 1,364 stage B cases, given their substantially greater genetic loading (Online Methods). For 'all' endometriosis, we observed the strongest signal for rs12700667 in an intergenic region on chromosome 7p15.2 ($P = 2.6 \times 10^{-7}$, OR = 1.22, 95%

CI 1.13–1.32; Table 2). As predicted from our quantitative genetic analyses, we observed stronger signals of association across the genome for stage B disease compared to 'all' endometriosis (Supplementary Fig. 3). The 7p15.2 signal for stage B endometriosis was considerably stronger, producing $P = 1.5 \times 10^{-9}$, OR = 1.38, 95% CI 1.24–1.53 (Table 2) for rs12700667 and $P = 6.0 \times 10^{-8}$, OR = 1.34, 95% CI 1.21–1.49 for the nearby SNP rs7798431 ($r^2 = 0.87$). A second strong association was found for rs1250248 (2q35) within *FN1* ($P = 3.2 \times 10^{-8}$) (Supplementary Table 3). Results for the SNPs rs12700667, rs7798431 and rs1250248 remained genome-wide significant after adjustment for multiple testing in the two non-independent genome-wide association analyses using permutation (Online Methods). Only one of the permuted genome-wide association analyses produced an independent P value less than that observed for rs12700667 ($P = 0.001$). The SNPs rs12700667 and rs7798431 lie in a narrow region of strong LD ($r^2 > 0.8$) that extends approximately 48 kb. Following imputation using 1000 Genomes Project and HapMap data (Fig. 2 and Supplementary Note) conditioning on the effect of rs12700667 in logistic regression analysis showed no other independent associations with 'all' or stage B endometriosis in the region.

Figure 2: Evidence for association with endometriosis across the chromosome 7 region following imputation using HapMap 3 and 1000 Genomes Project CEU and TSI reference panels.



Results for 'all' endometriosis are shown in **a**, and results for stage B endometriosis are shown in **b**. rs12700667 is represented by a purple diamond. All other SNPs are color coded according to the strength of LD (as measured by r^2) with rs12700667.

Table 2: GWAS, replication and meta-analysis results for rs12700667

In addition to the three genome-wide significant SNPs, we genotyped 70 SNPs that produced nominal evidence of association with 'all' ($P < 1.0 \times 10^{-4}$) or stage B endometriosis ($P < 1.0 \times 10^{-4}$ in stage B and $P < 1.0 \times 10^{-3}$ in 'all' endometriosis analyses; Online Methods) in an independent IEC dataset comprising 2,392 self-reported surgically confirmed cases from the Nurses' Health Study II (NHSII) and 2,271 controls from GWAS of breast cancer²⁰ and kidney function from the Nurses' Health Study (NHS) I and II. Stage information was not available for NHSII cases, but the proportion likely to have stage B disease has been estimated at approximately 40% (ref. 21), similar to that observed in the QIMR case set (Supplementary Table 1).

Association with 'all' endometriosis for the two SNPs on 7p15.2 was replicated in the US dataset, with $P = 1.2 \times 10^{-3}$, OR = 1.17, 95% CI 1.06–1.28 for rs12700667 and $P = 1.6 \times 10^{-3}$, OR = 1.17, 95% CI 1.06–1.28 for rs7798431 (Supplementary Table 3). There was no evidence (nominal $P \leq 0.05$) for replication of rs12540248 (*FN1*) or association with the remaining 70 SNPs (Supplementary Table 3).

Analysis of all 5,586 cases and 9,331 controls from the combined QIMR, Oxford and NHS cohorts further confirmed association between 'all' endometriosis and 7p15.2, producing $P = 1.4 \times 10^{-9}$, OR = 1.20, 95% CI 1.13–1.27 for rs12700667 and $P = 1.1 \times 10^{-7}$, OR = 1.18, 95% CI 1.11–1.25 for rs7798431 (Table 2). Although effect sizes from discovery datasets may be inflated²², the similarity of ORs for 'all' endometriosis in our discovery (GWAS) and replication datasets (Table 2) suggests this type of bias has not played a major role. Assuming the estimated OR of 1.20 and allele frequency of 0.74 for the rs12700667 A allele, a multiplicative risk model and a population prevalence of 8% (refs. 10,21,23), the estimated percentage of 'all' endometriosis variance explained by rs12700667 was 0.36, or 0.69% of the estimated 51% heritability of endometriosis⁹.

The associated SNPs are located in a ~924-kb intergenic region containing at least one noncoding RNA (AK057379), predicted transcripts and regulatory elements, and a miRNA (hsa-mir-148a) ~88 kb upstream of rs12700667. The closest gene, *NFE2L3*, which is highly expressed in placenta, is located ~331 kb downstream of rs12700667. Two endometriosis candidate genes, *HOXA10* and *HOXA11* (refs. 24,25), encoding members of the homeobox A family of transcription factors that play a role in uterine development, lie ~1.35 Mb downstream of this SNP.

Among reported candidate gene associations for endometriosis¹⁴, the only gene with $P < 10^{-3}$ for SNPs in the GWAS data was *PGR* on chromosome 11 (Supplementary Table 3), but the result for the SNP in this gene was not significant in the replication stage. A recent genome-wide association scan in Japanese women reported significant association of endometriosis with rs10965235 ($P = 5.8 \times 10^{-12}$, OR = 1.44), located on chromosome 9p21, and possible associations with rs13271465 on 8p22 and rs16826658 on 1p36 (ref. 26). The Japanese GWAS did not report our 7p15.2 signal among their 100 top SNPs followed up for replication, but with 1,423 cases and 1,318 controls, they would have had only 13% power to detect the effect of rs12700667 with $P \leq 1.8 \times 10^{-4}$ (Online Methods). We found no evidence for association with rs10965235 (which is monomorphic in individuals of European descent, reflecting the different genetic (ancestral) backgrounds between the studies) or any other SNP in LD ($r^2 > 0.5$ in the HapMap Japanese JPT population) in the QIMR and Oxford data (Supplementary Table 4). We also found no evidence of association with 8p22. We did find evidence for replication of rs7521902 on 1p36, which is close to *WNT4*, for both 'all' endometriosis ($P = 9.0 \times 10^{-5}$, OR = 1.16, 95% CI 1.08–1.25) and stage B cases ($P = 7.5 \times 10^{-6}$, OR = 1.25, 95% CI 1.13–1.38), with the stronger signal in stage B providing additional empirical evidence for the benefit in examining stage B cases. Importantly, a meta-analysis of the QIMR and Oxford 'all' endometriosis OR with the reported Japanese OR of 1.25 (95% CI 1.12–1.39) for rs7521902 produced a genome-wide significant P value of 4.2×10^{-8} (OR = 1.19, 95% CI 1.12–1.27). The frequency of the rs7521902 risk allele (A) was 0.57 and 0.51 in the Japanese GWAS cases and controls, respectively, and 0.26 and 0.24 in our combined GWAS cases and controls, respectively. *WNT4* is important for development of the female reproductive tract²⁷, ovarian follicle development and steroidogenesis^{28, 29}, making it a plausible biological candidate.

We have identified a new locus on chromosome 7p15.2 that is significantly associated with risk of endometriosis in women of European ancestry, and we confirm a previously reported suggestive association for SNPs close to the *WNT4* locus. Our analyses also demonstrate a higher genetic loading for moderate to severe (stage B) endometriosis, and consistent with these results, we observed the strongest association signals with stage B disease. Our predictive modeling demonstrates that there are additional common variants contributing to risk for this disease and that future larger studies enriched for laparoscopically-confirmed moderate to severe cases will be better powered to identify risk loci and aberrant pathways contributing to the development of endometriosis.

URLs.

ECR Browser, <http://ecrbrowser.dcode.org/>; SNPTESTv2, <http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html>; 1000 Genomes Project, <http://www.1000genomes.org/>; HapMap, <http://hapmap.ncbi.nlm.nih.gov/>.

Methods

GWAS samples and phenotyping.

For the current study, 2,351 surgically-confirmed endometriosis cases were drawn from individuals recruited by The Queensland Institute of Medical Research (QIMR) study³⁰ and a further 1,030 cases were obtained from individuals recruited by the Oxford Endometriosis Gene (OXEGENE) study. Controls consisted of 1,870 individuals recruited by QIMR^{31, 32} and a further 6,000 individuals provided by the Wellcome Trust Case Control Consortium 2 (WTCCC2) (Supplementary Note). Approval for the studies was obtained from the QIMR Human Ethics Research Committee and the Australian Twin Registry and the Oxford regional multi-centre and local research ethics committees. Informed consent was obtained from all participants prior to testing.

GWAS genotyping and quality control.

QIMR and Oxford cases and QIMR controls were genotyped at deCODE Genetics on Illumina 670-Quad (cases) and 610-Quad (controls) BeadChips (Illumina Inc). The WTCCC2 controls were genotyped at the Wellcome Trust Sanger Institute using Illumina HumanHap1M BeadChips.

Genotypes for QIMR cases and controls were called with the

Illumina BeadStudio software. Standard quality control procedures were applied as outlined previously (Supplementary Methods)³³. Following exclusions, 509,138 SNPs (2,270 cases and 1,870 controls) remained in the QIMR dataset. Oxford case and WTCCC2 control genotypes for all SNPs on the Illumina 670-Quad BeadChip were called using Illuminus³⁴. Following exclusions (Supplementary Note), 540,082 SNPs (924 cases and 5,190 controls) remained in the dataset. Post-quality-control genotype data from QIMR and Oxford were combined across 504,723 SNPs passing quality control measures in both datasets.

Proportion of variation explained by all markers and predictive modeling.

Using a new method¹⁸, we estimated the proportion of variation explained by all markers. As genotyping artifacts can severely bias these estimates, the SNP data were subjected to more restrictive quality control than that utilized for the genome-wide association analyses. SNPs with minor allele frequency <0.01, missing rates >0.001, *P* values for Hardy-Weinberg equilibrium <10⁻⁴, and non-autosomal SNPs were excluded. Individuals with missing rates >0.01, as well as one member of any pair of individuals with an estimated relationship >0.05, were also excluded. After quality control, 2,235 cases and 1,827 controls with 454,193 SNPs were used for analysis of the QIMR data, and 921 cases and 5,158 controls with 453,663 SNPs were used for analysis of the Oxford data (Supplementary Table 2). When combining the data, we first pruned the SNPs to a common set and excluded closely linked SNPs (with $r^2 > 0.5$ in sliding 50 SNP windows) using PLINK³⁵. Again, one member of any pair of individuals with an estimated relationship >0.05 was excluded. The combined analysis included 3,154 cases, 6,981 controls and 203,826 SNPs (Table 1).

Estimation of variation explained by all SNPs on the observed scale.

Pairwise realized relationships were estimated as described previously^{18, 36, 37}. Phenotypic observations (affected or unaffected, coded as 0 or 1) were modelled as a linear function of the sum of the additive effects due to all SNPs and residuals. For the combined analysis (QIMR and Oxford), cohort information was modeled as a covariate, which adjusts for the mean difference in the proportion of cases between the two cohorts. Variance components were estimated by residual maximum likelihood^{38, 39}.

The proportion of variation in case-control status explained by all SNPs simultaneously does not represent heritability in the conventional sense. Firstly, the observations are on the risk scale, whereas heritability estimates for disease from pedigree data are usually parameterized on an underlying unobserved liability scale. Secondly, the proportion of cases in the study is not the same as the proportion of cases in the population, so the estimate we obtained is with respect to a case-control population and not the population at large. Thirdly, conventional heritability from pedigree data captures the additive genetic variation due to all causal variants, whereas we captured only the variation due to causal variants tagged by SNPs on the arrays. Despite these caveats, the comparison of the proportion of variation estimates and the resulting P values from stage A and stage B cases remains valid because the test statistics would not change after scale transformation and ascertainment correction.

Case status prediction.

The aim of our prediction analysis was to evaluate the aggregate effects of many variants of small effect. We summarized variation across nominally associated loci into quantitative scores and related the scores to disease state in independent samples. Although variants of small effect (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 such as $P < 0.1$ (or, 10% of all SNPs), $P < 0.2$, etc. Using such thresholds, we defined large sets of allele-specific scores in the discovery sample of the Oxford dataset to generate risk scores for individuals in the target sample of the QIMR dataset. The term 'risk score' is used instead of 'risk', as it is impossible to differentiate the minority of true risk alleles from non-associated variants. In the discovery sample, we selected sets of allele specific scores for SNPs with $P < 0.01$, $P < 0.05$, $P < 0.1$, $P < 0.2$, $P < 0.3$, $P < 0.4$, $P < 0.5$ and $P < 0.75$. For each individual in the target sample, we calculated the number of score alleles they possessed, each weighted by the \log_{10} OR from the discovery sample. To assess whether the aggregate scores reflected endometriosis risk, we tested for a higher mean score in cases compared to controls. Logistic regression was used to assess the relationship between target sample disease status and aggregate risk score. Nagelkerke's pseudo R^2 was used to assess the variance explained. Autosomal SNPs with minor allele frequency < 0.01 and SNPs in high linkage disequilibrium were pruned, resulting in a set of 225,955 SNPs.

Genome-wide association analyses.

Although endometriosis is a condition exclusive to women, male and female controls were used in analyses of autosomal markers to maximize power, a method adopted previously in GWAS of breast cancer by the WTCCC^{40, 41}. No significant allele frequency differences were detected between male and female controls. Moreover, the genome-wide significant SNPs rs12700667 (7p15) and rs7521902 (*WNT4*) showed no heterogeneity between male and female controls ($P = 0.52$ and $P = 0.91$, respectively). Cochran-Mantel-Haenszel (CMH) tests of association with 'all' endometriosis or stage B alone were conducted in PLINK³⁵, with QIMR and Oxford data as different strata (to account for between population differences in baseline effect). Breslow-Day tests were conducted to check that the assumptions of the CMH test (that is, having the same effect size across strata) were true.

Permutation approach to correct for multiple testing.

To address the non-independence between the 'all' and stage B genome-wide association analyses, we utilized a permutation approach where case or control status was randomly shuffled separately within the QIMR and Oxford datasets to break the relationship between phenotype and genotype while retaining the relationship between 'all' and stage B endometriosis. Of the 1,000 permuted genome-wide associations, recording the minimum P value for each SNP after analysis of both 'all' and stage B cases, a $P \leq 6.5 \times 10^{-8}$ was obtained 50 times (genome-wide $P \leq 0.05$). Hence, rs12700667, rs7798431 and rs1250248 remained genome-wide significant after adjustment for multiple testing. Only one permuted genome-wide association produced an independent P value less than that observed for rs12700667 ($P = 0.001$, corrected for testing of both multiple markers and disease definitions).

Replication samples and genotyping.

Endometriosis cases ($n = 2,392$) for the replication samples were drawn from the US Nurses' Health Study (NHS) II^{21, 42}.

Replication controls were selected from two previous GWAS conducted in the NHSI and NHSII, including 1,142 postmenopausal, breast-cancer-free subjects from a breast cancer GWAS genotyped using the Illumina HumanHap 550 platform²⁰ and 1,129 subjects from a GWAS for kidney function⁴³ genotyped using Illumina 610 BeadChips. Quality control procedures have been described previously²⁰.

SNPs selected for replication were genotyped in the 2,392 NHS cases. Multiplex assays were designed using the Sequenom MassARRAY Assay Design software (version 3.0: Sequenom Inc.) and samples were genotyped using standard methods^{44, 45}. Post-laboratory quality control filtering was performed with PLINK³⁵.

Replication and meta-analyses.

GWAS SNPs reaching genome-wide significance were tested for replication in the NHS cohort. Also, to help direct further studies, we examined SNPs if they surpassed the following thresholds: (i) $P < 1.0 \times 10^{-4}$ in the 'all' endometriosis analysis (61 SNPs) or (ii) any SNPs not already included with $P < 1.0 \times 10^{-4}$ in the stage B analysis and $P < 1.0 \times 10^{-3}$ in the 'all' endometriosis analysis (14 SNPs). Genotype data were available for 2,271 NHS controls for 73 of these SNPs (Supplementary Table 3).

We estimated the association ORs and P values for the NHS cohort using PLINK³⁵ (Supplementary Table 3), and we performed CMH and Breslow-Day tests with the QIMR, Oxford and NHS datasets as distinct clusters. Meta-analysis for the QIMR, Oxford and Japanese²⁶ P values for 93 of the top 100 Japanese SNPs for which we had genotype data were conducted in GWAMA⁴⁶.

The power of the Japanese GWAS²⁶, including 1,423 endometriosis cases (unknown stage) and 1,318 controls, to detect an OR of 1.20 for a risk allele frequency of 0.80 (HapMapII JPT) of rs12700667, with a type I error of 1.8×10^{-4} (the threshold to select the top 100 SNPs for follow up in their replication dataset), was calculated using the Genetic Power Calculator⁴⁷.

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Competing financial interests

The authors declare no competing financial interests.

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Supplementary information

PDF files

1. Supplementary Text and Figures (944K)
Supplementary Note, Supplementary Tables 1–4
and Supplementary Figures 1–3

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