Replication and meta-analysis of previous genome-wide association studies confirm vezatin as the locus with the strongest evidence for association with endometriosis

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STUDY QUESTION: Is it possible to replicate the genetic association of single nucleotide polymorphisms (SNPs) rs13394619, rs4141819, rs7739264, rs17694933 and rs10859871 in five genetic loci previously identified as associated with endometriosis in an Italian Caucasian population?

SUMMARY ANSWER: SNP rs10859871 near the vezatin (VEZT) gene was found to be significantly associated with endometriosis in general while SNPs rs17694933 and rs4141819 were associated with Stage III/IV and ovarian disease, respectively.

WHAT IS KNOWN ALREADY: Endometriosis represents a complex disease in which the phenotypic manifestations are influenced by both genetic and environmental factors. Recent genome-wide association studies (GWASs) have allowed to identify some SNPs associated with the predisposition to the disease. A meta-analysis published in 2014 combined results from GWAS and replication studies showing that of the nine loci found to be associated with the disease in at least one of the studies, six (rs7521902, rs1270667, rs13394619, rs7739264, rs1537377 and rs10859871) remained genome-wide significant while two others (rs1250248 and rs4141819) showed borderline genome-wide significant association with more severe disease.

STUDY DESIGN, SIZE, DURATION: Allele frequencies of selected SNPs (rs13394619, rs4141819, rs7739264, rs17694933 and rs10859871) were investigated in 305 women with laparoscopically proven endometriosis, 285 laparoscopic controls and 2425 healthy, blood donor controls from the general population. A meta-analysis with previous data was also conducted.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 590 women who underwent endoscopic surgery were enrolled in the study and a blood sample was collected. After DNA extraction, genotype was obtained using Taq-Man pre-designed assay. Genotype data from healthy blood donor women were obtained from an existing genotype bank.

MAIN RESULTS AND THE ROLE OF CHANCE: A statistically significant association with endometriosis was found for SNP rs10859871, close to the VEZT gene, compared with both general population (odds ratio (OR) = 1.43, 95% confidence interval (CI): 1.20–1.71, P = 6.9 × 10^{-5}) and laparoscopic controls (OR = 1.58, 95% CI: 1.24–2.02, P = 2.1 × 10^{-4}). Meta-analysis with previous data confirmed the rs10859871 SNP as that with the strongest evidence for association with endometriosis (OR = 1.19, 95% CI: 1.15–1.24, P = 7.9 × 10^{-20}). A further meta-analysis conducted using data from Stage III–IV endometriosis resulted in stronger genome-wide significant effect sizes for four out of the five SNPs tested.

LIMITATIONS, REASONS FOR CAUTION: The inability to confirm all previous demonstrated associations considering all stages of endometriosis may be due to a lack of statistical power and differences in the definition of cases included.

WIDER IMPLICATIONS OF THE FINDINGS: The associations with the SNPs identified so far have been obtained with a relatively small...
sample size supporting a limited heterogeneity across the various datasets. This represents an important advance in the identification of genetic markers of this disease.

**STUDY FINDING/COMPETING INTEREST(S):** No funding to declare. The authors have no competing financial interests in relation to the content of this research paper.

**Key words:** vezatin / endometriosis / VEZT / replication / CDKN2B-AS1

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**Introduction**

Endometriosis has been long recognized as showing heritable tendencies. The first formal genetic study was conducted by Simpson et al. (1980) on 123 probands with histologically verified endometriosis. About 6% of female siblings had endometriosis and 8.1% mothers were affected. In contrast, only 1% of the patients’ husband’s first-degree relatives had endometriosis. These findings have been confirmed in subsequent studies in different populations (Painter et al., 1980). Overall, based on these studies and studies on twins, the proportion of disease variance due to genetic factors has been estimated at ~5% (Treloar et al., 1999) and the characteristic of familial aggregation makes endometriosis worthy of further genetic investigation. However, no candidate genes for the disease have been consistently replicated in different populations in previous association studies (Falconer et al., 2007).

Genome-wide association studies (GWASs) performed by different groups worldwide in this area of research should be viewed in this context. GWAS test associations for hundreds of thousands of individual single nucleotide polymorphisms (SNPs) selected to provide the maximum coverage of the genome and have been successful in the last years in detecting several variants associated with a range of human traits and common diseases (Manolio et al., 2009). These studies have provided a great amount of information suggesting high-confidence genotype–phenotype associations between specific genomic loci and several diseases, including diabetes, obesity, Crohn’s disease and hypertension (Vigano et al., 2012).

Two GWAS have been published in 2010 on Japanese datasets (Adachi et al., 2010; Uno et al., 2010). The first GWAS in women of European ancestry was conducted by the International Endogene Consortium involving Australian and UK datasets with an independent replication in a US dataset (Painter et al., 2011). A fourth GWAS in women of European ancestry was published in 2013 on US cases and controls (Albertsen et al., 2013). Two independent replication studies were also published, one from our group (Pagliardini et al., 2013), showing the genotyping results of some of the variants identified by GWAS (Sundqvist et al., 2013). Meta-analysis was applied to combine GWAS results with those from replication studies and published in 2014 (Rahmioglu et al., 2014). Results have shown that of the nine loci found to be associated with the disease in at least one of the studies, six remained genome-wide significant including rs7521902 close to WNT4, rs1270667 on 7p15.2, rs13394619 in Growth regulation by estrogen in breast cancer 1 (GREB1), rs7739264 near Inhibitor of DNA binding 4 (ID4), rs1537377 near Cyclin-dependent kinase inhibitor 2B antisense RNA (CDKN2B-AS1) and rs10859871 near vezatin (VEZT). In addition to these six loci, two others showed borderline genome-wide significant association with Stage III and IV disease, including rs1250248 in fibronectin I (FN1) and rs4141819 on 2p14 (Rahmioglu et al., 2014).

In support of the existence of a limited heterogeneity across the various datasets, it should be considered that our first replication study (Pagliardini et al., 2013) in an Italian population was indeed able to confirm association for three out of four of the loci considered, thus contributing to a higher significance of the meta-analysis results. In particular, we have been the first replication study confirming FN1 / rs1250248 as a susceptibility locus for severe endometriosis and we have further confirmed that the variant close to WNT4, a critical regulator of post-natal uterine development, was significantly associated with endometriosis. Finally, we have provided the first evidence of association of the CDKN2B-AS1 locus with endometriosis also in the Caucasian population in addition to the previously described Asian population (Pagliardini et al., 2013).

Based on these results, the present study aimed at evaluating loci showing genome-wide significant association in the previous meta-analysis by Rahmioglu et al. (2014) but not already investigated in our previous replication in an Italian population (Pagliardini et al., 2013). Surprisingly, to date the samples sizes of the endometriosis GWAS (n = 6636 GWAS individuals and n = 4870 samples for replications) are at the lower end of GWAS in other complex disease fields (e.g. n = 47 000 GWAS individuals and n = 94 000 replication samples for Type 2 diabetes) (Visscher et al., 2012). A larger sample size for genome-wide meta-analysis is predicted to provide further consistency to the loci identified so far. It is in this context that the present study was designed.

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**Materials and Methods**

**Subjects**

A total of 390 women were enrolled at the endoscopic surgical service of the Obstetrics and Gynecology Unit of the San Raffaele Scientific Institute, Milan, Italy, between December 2009 and December 2010 and endometriosis was laparoscopically documented in 305 (52%). DNAs from this cohort have been genotyped for different SNPs and results have been reported in a previous paper (Pagliardini et al., 2013). Stage of the disease was found to be minimal or mild (Stages I–II) in 84 cases (28%) and moderate to severe (Stages III–IV) in 221 cases (72%), staged according to the revised classification system of the American Society for Reproductive Medicine (1997). The manifestation of the disease at the ovarian site (cysts and lesion) was observed in 210 (69%) women. Deep endometriosis, defined as lesions infiltrating to a depth of at least 5 mm beneath the peritoneal surface, was observed in 175 (57%) cases. The 285 women in whom laparoscopy evidenced no endometriosis served as the control group (laparoscopic controls). Specifically, leiomyoma was observed in 60 women (21%), non-endometriotic benign cysts in 139 women (49%), pelvic inflammatory disease in 14 women (5%), pelvic adhesions in 18 women (6%), uterine malformations in 22 women (8%) and 50 women presented a regular pelvis (18%) (Some patients had more than 1 pathological finding).

A second group of controls composed of healthy female blood donors was obtained from the genotype bank of Institute Auxologico Italiano (general
population controls, n = 2425). All individuals studied were of Italian Caucasian origin. Approval for this study was granted by the local Human Institutional Investigation Committee.

SNP selection
We genotyped the five SNPs that reached a P-value of < 10⁻⁷ in at least one of the analysis groups deriving from the meta-analysis by Rahmioglu et al. (2014) and that have not been already genotyped in our previous study (Pagliardini et al., 2013). The design of a custom probe for rs1537377 was not feasible due to the close proximity of another SNP (rs2184059, located within two bases away). Therefore, we genotyped the SNP rs17694933 located in the same block of linkage disequilibrium (LD) (r² = 0.93). Ultimately, five SNPs—rs13394619, rs4141819, rs7739264, rs17694933 and rs10859871—were selected for this replication study. Details and results for the investigated loci in relation to the association with endometriosis reported by Rahmioglu et al. (2014) are summarized in Supplementary data, Table SI.

SNP genotyping
Whole-blood samples were drawn from each patient before surgery and collected in an EDTA-containing tube. Genomic DNA was extracted from blood with the Nucleon BACC2 kit (Amsherms Biosciences, Munich, Germany) according to the manufacturer’s protocol. Surgical samples were genotyped using a Taq-Man pre-designed SNP genotyping assay from Applied Biosystem, Inc. (ABI, Foster City, CA, USA). Taq-Man allelic discrimination analyses were performed on an ABI 7900HT genotyping analyzer using 11 μl of genomic DNA (1.8 ng/μl) following manufacturer’s instructions. Genotypes were determined using the Taq-Man genotyping software SDS (v2.2, ABI, Foster City). To validate the genotyping assay, 5% randomly selected samples were sequenced.

Controls from the general population were genotyped using Illumina 660W-Quad BeadChip (Illumina, Inc., San Diego, CA, USA). Quality control of the data was performed according to the protocol described by Anderson et al. (2010).

Statistical analysis
LD was assessed using the Haploview software (v.4.2, Broad Institute, Cambridge, MA, USA). Hardy–Weinberg equilibrium (HWE) was tested separately in laparoscopic and general population controls using the goodness-of-fit χ² test. PLINK (v.1.07, http://pngu.mgh.harvard.edu/~purcell/plink/) was used to perform the standard case/control association analysis. Meta-analysis was performed using METAL (http://www.sph.umich.edu/csg/abecasis/metal/), S february 2015, date last accessed). It was performed with data from the study by Rahmioglu et al. (2014) using a fixed-effect model where the effect size estimates (β-coefficients) are weighted by their estimated standard errors. For SNPs that showed evidence of effect heterogeneity in the meta-analysis by Rahmioglu et al. (2014), P-values calculated with a random-effect model were included in the analysis. Heterogeneity of allelic effects across studies was examined using Cochran’s Q-test (Cochran, 1954). Given the unequal number of cases and controls in study populations, we utilized the effective sample size for meta-analysis, where Neff = 4/(1/Ncases + 1/Ncontrols) (Willer et al., 2010). Odds ratios (ORs) with 95% confidence intervals (CIs) were used to measure the strength of the association. P-values of <0.05 were considered significant. Statistical power analysis was performed using G*power 3 (http://wwwpsycho.uni-duesseldorf.de/abteilungen/aap/gpower3/, S February 2015, date last accessed). Considering that our case population was enriched for severe phenotypes (Stage III/IV endometriosis) and that greater ORs were previously observed for most of the variants considered for these phenotypes, we expected larger effect sizes than those reported in the literature (Rahmioglu et al., 2014). This has already been confirmed in our previous study (Pagliardini et al., 2013) in which we observed effect sizes greater than those reported by other authors (Uno et al., 2010; Painter et al., 2011). Based on these concepts, sample size was a priori calculated in order to detect an arbitrary defined effect OR of 1.4, assuming type I and II error of 0.05 and 0.2, respectively. Calculation for the SNP with the lower allele frequency (rs4141819) resulted in a sample size of at least 279 cases and 2214 controls.

Results
Genetic association of the SNPs with endometriosis
No significant deviation of genotype frequency from HWE was detected for the analyzed SNPs in laparoscopic and general population controls.

Allele frequencies and ORs (95% CI) for associations of rs13394619, rs4141819, rs7739264, rs17694933 and rs10859871 with endometriosis and the results of the meta-analysis with data from the study by Rahmioglu et al. (2014) are presented in Table I.

We found a statistically significant association between rs10859871, close to VEZT gene, and endometriosis compared with the general population (OR = 1.43, 95% CI: 1.20–1.71, P = 6.9 × 10⁻⁵). This association resulted in a higher effect size (OR) when risk allele C frequency of rs10859871 was compared with laparoscopic controls (OR = 1.58, 95% CI: 1.24–2.02, P = 2.1 × 10⁻⁴). No other significant association has been detected for the remaining four SNPs, although the direction of the effect of all SNPs is the same reported in the meta-analysis by Rahmioglu et al. (2014).

When the allele frequencies were used to evaluate association with the different phenotypes of endometriosis (Table II), a nominally significant genetic association was found for two other SNPs compared with the general population. Risk allele A of rs17694933 at 9p21.3 was associated with more severe stages of endometriosis with an OR of 1.25 (95% CI: 1.02–1.54, P = 3.3 × 10⁻²). Conversely, the manifestation of the disease at the ovarian site showed an increased allele frequency for risk allele C of rs4141819 (OR = 1.26, 95% CI: 1.02–1.55, P = 3.3 × 10⁻²). Interestingly, the use of a dominant model to describe the association between rs4141819 and endometriosis in the ovarian site results in a higher OR of 1.52 (95% CI: 1.13–2.06, P = 5.6 × 10⁻³).

Meta-analysis with previous data
Results for meta-analysis with datasets from Rahmioglu et al. (2014) showed no evidence of heterogeneity, with all the SNPs showing consistent directions of effect between studies (Tables I and III). The SNP rs17694933, located in the CDKN2B-AS1 locus, was different from the one presented in the meta-analysis published by Rahmioglu et al. (2014) and for this reason was excluded from our analysis.

Results of the meta-analysis for all stages of endometriosis are reported in Table I and confirm a genome-wide significant association for SNPs rs10859871 (OR = 1.19, 95% CI: 1.15–1.24, P = 7.9 × 10⁻²⁰) and rs7739264 (OR = 1.11, 95% CI: 1.08–1.14, P = 4.6 × 10⁻¹¹). Conversely, association with endometriosis for SNPs rs13394619 and rs4141819 failed to reach a genome-wide significant P-value after inclusion of our data.

An additional meta-analysis was conducted with the 'Stage III-IV enriched' dataset from Rahmioglu et al. (2014) that excluded from the analysis all the subjects classified as 'Stages I–II', while keeping all the 'unknown stage' subjects in the analysis. Results reported in Table III...
**Table I** Allele frequencies comparison between endometriosis patients and controls and meta-analysis of the present replication study and previous studies.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Allele&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nearest gene</th>
<th>Present study</th>
<th>Meta-analysis&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td></td>
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<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>rs13394619</td>
<td>G/A</td>
<td>GREB1</td>
<td>Laparoscopic</td>
<td>0.458</td>
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<td></td>
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<td></td>
<td>General population</td>
<td>0.458</td>
</tr>
<tr>
<td>rs4141819</td>
<td>C/T</td>
<td>Intergenic</td>
<td>Laparoscopic</td>
<td>0.342</td>
</tr>
<tr>
<td>rs7739264</td>
<td>C/T</td>
<td>ID4</td>
<td>Laparoscopic</td>
<td>0.562</td>
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<td></td>
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<td>General population</td>
<td>0.562</td>
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<tr>
<td>rs17694933</td>
<td>A/G</td>
<td>CDKN2B-AS1</td>
<td>Laparoscopic</td>
<td>0.422</td>
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<td>General population</td>
<td>0.422</td>
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<tr>
<td>rs10859871</td>
<td>C/A</td>
<td>VEZT</td>
<td>Laparoscopic</td>
<td>0.462</td>
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<td></td>
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<td>General population</td>
<td>0.462</td>
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SNPs, single nucleotide polymorphisms; RAF, risk allele frequency; OR, odds ratio; CI, confidence interval; Q, Cochran’s Q-test results; GREB1, growth regulation by estrogen in breast cancer 1; ID4, inhibitor of DNA binding 4; CDKN2B-AS1, cyclin-dependent kinase inhibitor 2B antisense RNA 1; VEZT, vezatin.

<sup>a</sup>Minor allele/major allele.

<sup>b</sup>Odds ratio relative to risk allele.

<sup>c</sup>With data from Rahmiglu et al. (2014).
showed an increased OR for all the four SNPs when compared with the meta-analysis including all stages of endometriosis. Moreover, all the $P$-values for the associations are under the genome-wide significant threshold of $5 \times 10^{-8}$.

**Discussion**

The results of this replication study in an Italian population confirms rs10859871, the SNP located 17 kb upstream of VEZT, as significantly associated with endometriosis and the one with the strongest association among the SNPs identified by GWAS. The meta-analysis including results from previous studies allowed to demonstrate a genome-wide significance at results from previous studies. The meta-analysis including associated with endometriosis and the one with the strongest association among the SNPs identified by GWAS. The meta-analysis including

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<th>Table II</th>
<th>Association of the SNPs with sub-phenotypes of endometriosis.</th>
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<td>SNPs</td>
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<td>rs13394619</td>
<td>GREB1</td>
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ORs are calculated for risk alleles of Table I. Data in bold are significant results.

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<th>Table III</th>
<th>Meta-analysis with previous published data for Stage III and IV endometriosis.</th>
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<td>rs10859871</td>
<td>VEZT</td>
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$^a$General population controls.

$^b$With data from Rahmioglu et al. (2014) for ‘Stage III-IV enriched’ dataset.

Q, Cochran’s Q-test results.

The VEZT locus encodes the transmembrane protein VEZT which has been identified as a component of the E-cadherin-catenins complex at adherens junctions (Hyenne et al., 2007). Zygotic-null mouse embryos die at implantation because of a defect in cell–cell adhesion suggesting that VEZT is essential for adhesive forces necessary for changes in cell shape and/or movements during embryogenesis. It has been suggested that the lack of VEZT would lead to unstable interactions between the E-cadherin-catenins complex and the actin cytoskeleton at adherens junction. Alternatively, VEZT can enter the cell nucleus regulating the expression of targeted genes modulating cell adhesion and invasion (Hyenne et al., 2007). Other information regarding this gene is limited. More recently, it has been postulated that VEZT acts as a putative tumor suppressor gene, targeting cell migration and invasion genes (Miao et al., 2013). Some groups including ours have investigated the involvement of cell adhesion, migration and invasion in the pathogenesis of endometriosis. Alterations in the expression pattern of specific adhesion molecules such as integrins (Lessey et al., 1992; Starzinski-Powitz et al., 1999) and cadherins (Gaetje et al., 1997) have been observed in both eutopic and ectopic cells from women with endometriosis. Cytoskeletal-actin dynamics affecting cell migration have been demonstrated to be differentially regulated by steroid hormones in patients with and without endometriosis (Gentilini et al., 2010). Therefore, it is not surprising that a variant of a gene involved at the junction of intercellular adhesions and actin dynamics may be associated with endometriosis.

The present study also confirms the CDKN2B-AS1 locus as significantly associated with the disease (Uno et al., 2010; Nyholt et al., 2012; Pagliardini et al., 2013; Rahmioglu et al., 2014). The SNP evaluated in the present study is located 43 kb 3’ to the transcriptional end site of CDKN2B-AS1 and it is not correlated with the rs133049 SNP that we have been previously identified in this region (Pagliardini et al., 2013).
studies are needed to clarify the potential role of this gene variant in GREB1 expression according to the stage of the disease. Further, the LD metrics between the two SNPs present an \( r^2 < 0.001 \) and a \( D' = 0.011 \), confirming that there may be two independent genetic risk factors near the CDKN2B-AS1 locus (Nyholth et al., 2012).

It is interesting to observe that the two SNPs rs1250248 in FN1 and rs4141819 on 2p14, previously found to have borderline genome-wide significant associations with Stage III/IV endometriosis (Painter et al., 2011; Nyholth et al., 2012), have both been observed to be significantly associated with ovarian endometriosis in our replications (Pagliardini et al., 2013). Variability in phenotypic characterization of endometriosis cases between studies is likely to contribute to the heterogeneity in findings and to the dilution of the strength of association. None of the GWAS published so far was able to provide the proportion of the ovarian sub-phenotype of the disease. Therefore, our replication can substantially contribute in this regard. As a matter of fact, considering the high prevalence of ovarian endometriosis in our cohort, the present meta-analysis for Stage III/IV has demonstrated a genome-wide significant association also for rs4141819 on 2p14 that showed only a borderline association in the meta-analysis published by Rahmioglu et al. (2014).

Fibronectin is fundamental for the structure of a corpus luteum (Irving-Rodgers et al., 2006). The newly formed luteal tissue is rich in fibronectin and relatively low in collagen I and as the tissue become more organized, the abundance ratio of collagen to fibronectin reverses to produce a firm tissue. The role of fibronectin would be to define the early structural arrangement of the tissue and to provide a guiding scaffold for subsequent collagen deposition. One of the theory for endometrioma formation implies that the ovoid would adhere to the broad ligament because of the presence of endometriotic implants with the subsequent formation of adhesions. Afterward, the attached implant would guide ovulation to occur in this site (inflammatory molecules are involved in the mechanisms of follicle dehiscence) and endometriotic cells can then invade the newly formed corpus luteum that would not undergo resorption (Vercellini et al., 2009; Viganò et al., 2013; Sanchez et al., 2014). Thus, a possible alternative source of the entrapped blood would be consequent to the formation of a non-reabsorbed cystic corpus luteum. Based on this theory, the possible involvement of a FN1 gene variant in endometrioma formation seems to acquire biological plausibility. In contrast, the role for rs4141819 on 2p14 in association with ovarian endometriosis is totally unknown. This variant is located in an intronic region of the long non-coding RNA AC007422.1 that, similarly to CDKN2B-AS1, is likely to have a regulatory role in gene expression (Rahmioglu et al., 2014).

In contrast with Rahmioglu et al. (2014) we have been unable to find a nominal association for rs13394619 in GREB1 with endometriosis. Moreover, our meta-analysis with previous studies failed to obtain a genome-wide significant association considering all stages of endometriosis while a genome-wide significant association was found for the more severe forms of the disease. We cannot exclude that this may be due to a lack of statistical power to demonstrate all the hypothesized associations. This represents a limitation of the present study. Interestingly, this gene of unknown function has been suggested to play a role in the estradiol-dependent proliferation of breast and endometrial cancer cells and expression was found to be increased in both eutopic and ectopic tissue from women with peritoneal endometriosis by Pellegrini et al. (2012). Unfortunately, these authors did not evaluate GREB1 expression according to the stage of the disease. Further studies are needed to clarify the potential role of this gene variant in endometriosis development.

Our meta-analysis considering more severe forms of the disease confirms genome-wide association with endometriosis for SNPs in four of the five genetic loci analyzed in an Italian population, with a stronger effect size compared with the general endometriosis population.

Endometriosis is a disease characterized by a still poorly defined phenotype. The disease stage depends on the type (cysts, implants and nodules), location (ovary, peritoneum, bladder, ureter etc.), appearance and depth of invasion of the lesions that can vary greatly among patients (Nsolle and Donnez, 1997; Fauconnier and Chapron, 2005; Chapron et al., 2006; Farquhar, 2007). Staging may be useful in determining the overall burden as well as the management of the disease but it correlates neither with pain symptoms nor with infertility (Vercellini et al., 2007; Viganò et al., 2009; de Ziegler et al., 2010; Stratton and Berkley, 2011). The basis of such a huge phenotypic heterogeneity in endometriosis is still poorly clarified but may have a genetic origin. GWAS studies including data related to sub-phenotypes of the disease should be able to clarify some mechanisms underlying genotype–phenotype relationships.

It is very interesting to observe that the associations identified so far have been obtained with a relatively small sample size. In any case, assuming a population prevalence for endometriosis of \(~5\%\) (Vercellini et al., 2014), these loci together account for \(< 2\%\) of all endometriosis susceptibility. Therefore, they are presently not useful in predicting disease risk.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Authors’ roles**

A.M.D.B., M.C. and P.V. contributed to study concept and design. D.G., L.P. and A.M.S. performed DNA extraction, SNP genotyping and statistical analysis. L.P. and P.V. contributed to the drafting of the manuscript and critical discussion. A.M.D.B., M.C., D.G. and A.M.S. revised critically the manuscript. All the authors approved the final version of the article.

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**Conflict of interest**

None declared.

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