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ORIGINAL ARTICLE

The mid-secretory endometrial transcriptomic landscape in endometriosis: a meta-analysis

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STUDY QUESTION: Do women with endometriosis have a different endometrial gene expression profile at the time of embryo implantation than women without endometriosis?

SUMMARY ANSWER: The endometrial gene expression profile of women with endometriosis differs from that of women without endometriosis at the mid-secretory phase, although the differences are small.

WHAT IS KNOWN ALREADY: About 50% of women with endometriosis suffer infertility. Several molecular studies have suggested impaired endometrial receptivity in women with endometriosis, while others have detected no dysregulation of endometrial receptivity. Nevertheless, the previous endometrial transcriptome studies comparing women with and without endometriosis have been performed in small sample size with limited statistical power. We set out to systematically search and compile data of endometrial gene expression signatures at the receptive phase in women with endometriosis versus control women. Based on the obtained data, we conducted a meta-analysis of differentially expressed genes in order to raise the power of the analysis for identifying the molecular profiles of receptive phase endometria in endometriosis.

STUDY DESIGN, SIZE, DURATION: A systematic literature search was conducted up to February 2022 following PRISMA criteria and included PubMed, Cochrane and Web of Science databases. For the systematic search, the term 'endometriosis' was paired with the terms 'transcriptomics', 'transcriptome', 'gene expression', 'RNA-seq', 'sequencing' and 'array', by using the Boolean operator 'AND' to connect them. Articles written in English were screened and interrogated for data extraction.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A meta-analysis was performed on the selected studies to extract the differentially expressed genes described at the mid-secretory phase in women with endometriosis versus women without endometriosis in natural cycles, using the robust rank aggregation method. In total, transcriptome data of 125 women (78 patients and 47 controls) were meta-analysed, with a special focus on endometrial receptivity-specific genes based on commercial endometrial receptivity tests.

MAIN RESULTS AND THE ROLE OF CHANCE: In total, 8 studies were eligible for the quantitative meta-analysis, gathering transcriptome data from the mid-secretory phase endometria of 125 women. A total of 7779 differentially expressed transcripts between the study groups were retrieved (3496 up-regulated and 4283 down-regulated) and were meta-analysed. After stringent multiple correction, there was no differential expression of any single molecule in the endometrium of women with endometriosis versus controls, while enrichment analysis detected that the pathways of chemotaxis and locomotion are dysregulated in endometriosis. Further analysis of endometrial receptivity-specific genes highlighted dysregulation of *C4BPA*, *MAOA* and *PAEP* and enrichment of immune and defence pathways in women with endometriosis.

LIMITATIONS, REASONS FOR CAUTION: Most of the studies included into the meta-analysis were relatively small and had different study designs, which might have contributed to a bias.

WIDER IMPLICATIONS OF THE FINDINGS: The current meta-analysis supports the hypothesis that endometrial receptivity is altered in women with endometriosis, although the changes are small. The molecules and pathways identified could serve as future biomarkers and therapeutical targets in detecting and treating endometriosis-associated infertility.

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Key words: endometriosis / endometrium / infertility / meta-analysis / transcriptomics

WHAT DOES THIS MEAN FOR PATIENTS?

This study investigates whether the gene expression profile of the endometrium (the inner lining of the uterus) of women with endometriosis is different from that of control women during the phase of the menstrual cycle when the uterus is receptive for embryo to implantation. Our extensive systematic review and meta-analysis identified endometrial receptivity-associated genes and molecular pathways that seem to be altered in women with endometriosis. The study findings could help to explain endometriosis-associated infertility in women suffering from this common gynaecological disease and could lead to the development of molecular biomarkers for detecting and treating infertility in endometriosis.

Introduction

Endometriosis is a debilitating gynaecological condition that affects ~10% of women of reproductive age and is characterized by the implantation of endometrial tissue in ectopic locations (Zondervan *et al.*, 2018). Among the main symptoms of endometriosis, pelvic pain, dysmenorrhoea and infertility are the most prevalent, with evidence of impaired fertility in up to 50% of affected women (Giudice and Kao, 2004; Practice Committee of the American Society for Reproductive Medicine, 2012). One of the suggested reasons for endometriosis-associated infertility is diminished endometrial receptivity and defective embryo implantation (Brosens *et al.*, 2012; Altmäe and Aghajanova, 2015; Lessey and Kim, 2017; Horton *et al.*, 2019).

Accumulating evidence suggests that endometriosis has a detrimental reproductive impact in both natural as well as assisted reproduction, where the potentially dysfunctional endometrium, aberrant uterine contractility and affected endometrium–myometrium interface could hinder embryo implantation (Horton *et al.*, 2019). In addition, different pathological processes involving inflammation, immune modulation, aberrant angiogenesis, oxidative stress, extracellular matrix remodelling, genetic and epigenetic changes could impact endometrial receptivity and implantation process in women with endometriosis (Gupta *et al.*, 2008; Kokcu, 2013; Vigano *et al.*, 2015). However, there is no consensus about whether endometrial receptivity is dysregulated in endometriosis and which molecular mechanisms are involved.

Several studies focusing on single molecules have observed alterations in the expression levels of different genes and proteins in the endometria of women with endometriosis (Giudice *et al.*, 2002; Wei *et al.*, 2009; May *et al.*, 2011). Nevertheless, the results obtained are controversial and lack confirmation and validation. With the advancement of '-omics' technologies, several studies have investigated the gene expression profile of the whole genome (i.e. transcriptomics) in eutopic endometria from women with endometriosis (Giudice, 2003; Fassbender *et al.*, 2012; Altmäe *et al.*, 2014; Miravet-Valenciano *et al.*, 2017; Saare *et al.*, 2017; McKinnon *et al.*, 2018; Poli-Neto *et al.*, 2020). Regardless of the long lists of differentially regulated genes identified in the whole genome expression analyses, the studies are heterogeneous, performed on limited sample size, and lack power, consensus and validation. Therefore, whether endometrial transcriptome is dysregulated in the endometrium in the receptive phase in endometriosis remains an open debate.

We set out to perform a systematic literature search followed by a meta-analysis of the endometrial transcriptome at the mid-secretory phase eutopic endometria in women with endometriosis in comparison with endometria from women without the disease in order to raise the power in identifying endometrial transcriptome profiles.

Materials and methods

Search of the literature and data extraction

A systematic search of the literature was performed using PubMed, Cochrane and Web of Science databases up to February 2022 by two researchers (S.A. and E.V.) independently and in agreement with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Page *et al.*, 2021). For the systematic search,

the term 'endometriosis' was paired with the terms 'transcriptomics', 'transcriptome', 'gene expression', 'RNA-seq', 'sequencing' and 'array', by using the Boolean operator 'AND' to connect them.

Titles of the papers were read to extract information of the potentially eligible abstracts, which were then carefully evaluated for further full-text evaluation. The reference lists of review articles and relevant studies were explored manually to identify other potentially eligible studies. No restrictions were applied. A detailed protocol for this systematic review was registered in PROSPERO under the title 'Endometrial transcriptome in women with endometriosis compared with women without endometriosis during the window of implantation: systematic review and meta-analysis' and can be accessed at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020122054.

Abstracts of all the retrieved articles were read for the selection of eligible studies, and the full text of each potentially suitable article was evaluated. In the final step, we restricted the inclusion criteria only to original experimental studies concerning the endometrial transcriptome (cDNA microarray or RNA-sequencing techniques) in women with endometriosis in the mid-secretory phase of the menstrual cycle compared with control women without endometriosis from the same phase of the cycle. Articles focusing on the study of other phases of the menstrual cycle or including pathological conditions other than endometriosis were not included in the final list of articles.

For all eligible studies, the lists of differentially expressed genes in the mid-secretory phase in eutopic endometrial tissue of women with endometriosis versus normal endometrium from control women were extracted directly from the publication. When the gene lists were not available, the authors were contacted. In preparation for the subsequent analyses, all the gene lists were standardized to a common nomenclature system using the official gene symbols. Therefore, all lists that had other gene identifiers (GenBank IDs or Affymetrix array probes IDs, mainly) were converted into the official gene symbols by using the g:Convert tool in g:Profiler database (https://biit.cs.ut.ee/gprofiler/convert; Raudvere *et al.*, 2019). All the array probes unable to be converted to official gene symbol were removed from the subsequent analyses. Finally, the lists of dysregulated genes were ranked by the absolute value of their fold changes (FCs). For the genes with duplicated values of FC, only the highest absolute value of the FC remained.

Meta-analysis

The robust rank aggregation (RRA) method (RRA package v.1.1) implemented in R (version 3.5.1) was used for the meta-analysis of the ranked gene lists (Kolde *et al.*, 2012). The total number of official gene symbols ranked by their FCs in each study was used as an input. The RRA method assigns a significance ρ (rho) score for each transcript, which is used to order the genes based on that value (Kolde *et al.*, 2012), resulting in a list of prioritized genes in accordance to the representation each gene has in each list of differentially expressed genes. This parameter is not itself a *p*-value and therefore has to be corrected. Hence, to adjust for multiple testing, a false discovery rate (FDR) correction was calculated following the Benjamini–Hochberg procedure using the *p*.adjust function included in R package *stats*. All the lists involved in the analysis reflect expression in the receptive phase of the menstrual cycle in the endometriosis group compared with the control group.

Next, we focused on the known endometrial receptivity genes by extracting gene lists from three commercially available endometrial receptivity tests: (i) the endometrial receptivity array (ERA; Igenomix, Spain) which includes 238 genes (Díaz-Gimeno *et al.*, 2011); (ii) the ER Map®/ER Grade® test (iGLS, Spain) with focus on the 25 receptivity-sensitive genes (Enciso *et al.*, 2018); and (iii) the beREADY® test (Competence Centre on Health Technologies, Estonia), integrated by a gene list of 57 genes (based on meta-analysis by Altmäe *et al.* (2017)). Every dataset included in our meta-analysis was assessed for any differentially expressed endometrial receptivity-specific transcript, and independently across the intersection between each receptivity test and the meta-analysed studies (ERA test versus studies; ER Map®/ER Grade® test versus studies; and beREADY® test versus studies). In preparation for the RRA method, the FC of the differentially expressed transcripts belonging to the receptivity-specific genes were extracted, and gene symbols were ranked according to that value.

Enrichment analyses

The functional enrichment analysis for Gene Ontology (GO) terms and biological pathways was performed using *gprofiler2* package implemented in R (Raudvere *et al.*, 2019) with Benjamini–Hochberg FDR multiple testing correction method applying a significance threshold at 0.05. The 'ordered query' option was applied. As data sources, the overrepresented signalling pathways were obtained by using GO for discovery of Biological Processes (BP), Molecular Functions (MF) and Cellular Components (CC), the Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome (REAC) databases. Functional enrichment analysis was performed for the ranked list of genes obtained after the application of the RRA method, and for the genes in the meta-analysed dataset specifically involved in endometrial receptivity.

Data mining of candidate genes

High-throughput experiments usually generate large sets of potential candidates among which only a few could be interpreted as truly relevant to the phenotype of study (Tranchevent *et al.*, 2011). In our study, both web and desktop tools were applied to further investigate the main characteristics of the proposed candidates. DisGeNET, a discovery platform of genetic associations for human diseases (Bauer-Mehren *et al.*, 2010; Piñero *et al.*, 2020) was employed to interrogate for gene variants of endometriosis and also to check the diseases that have been reported in association with the candidate genes which arose from our study using the gene–disease associations and variant–disease associations tools, respectively (Bauer-Mehren *et al.*, 2011; Piñero *et al.*, 2020). Finally, and following the analyses based on network approaches, GeneMANIA plugin implemented in Cytoscape software was used to characterize and predict the interactions among the gene sets of interest (Warde-Farley *et al.*, 2010). The complete pipeline of analysis we followed in this study is summarized in Fig. 1.

Results

Systematic literature search, meta-data creation and meta-analysis

Out of the 155 total eligible studies obtained from the systematic literature search, 8 studies focusing on the analysis of the gene expression

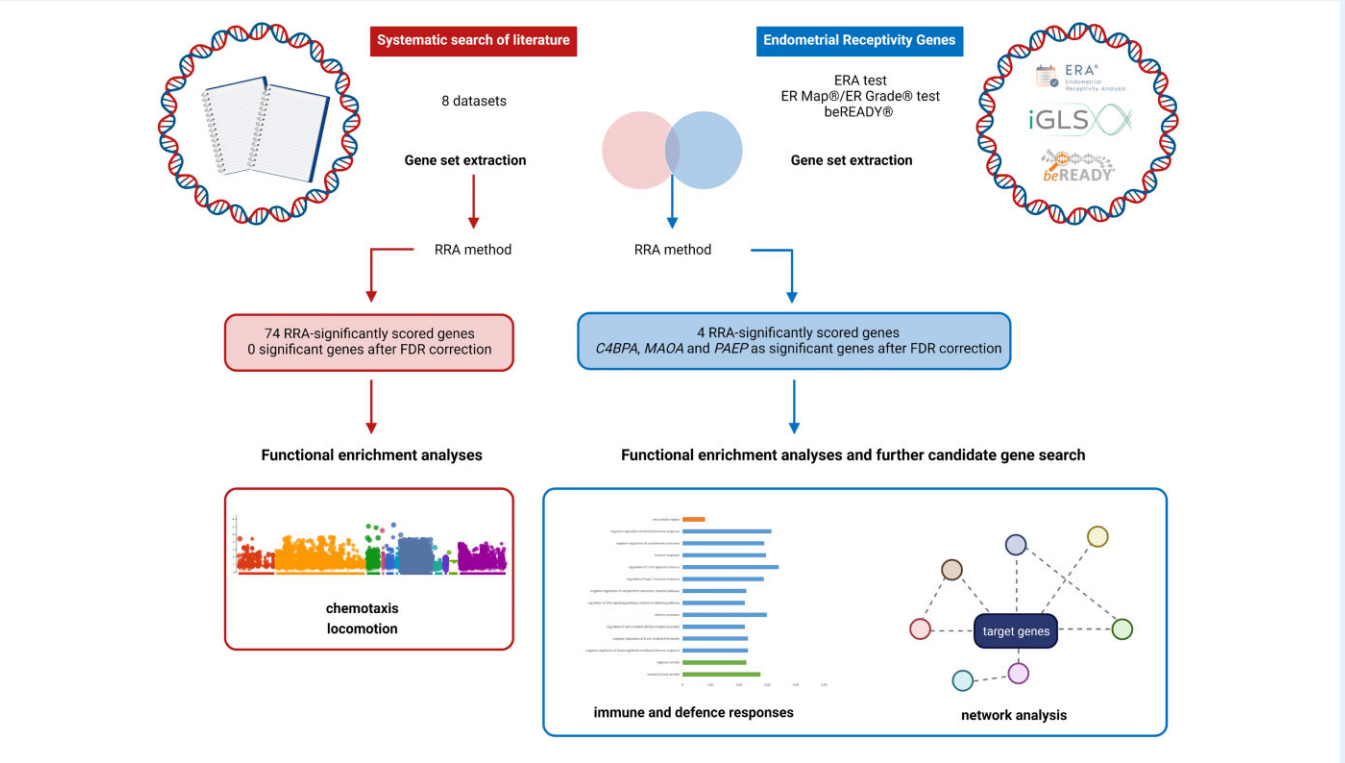

Figure 1. Study design and the main results obtained. The initial systematic literature search resulted in eight studies suitable for the data extraction. In parallel, data from commercial endometrial receptivity tests were extracted and used to generate a dataset of endometrial receptivity-specific genes. Both independent datasets and their intersection were meta-analysed using the robust rank aggregation (RRA) method. Obtained results were utilized to perform functional enrichment analyses and further candidate gene search. FDR, false discovery rate. Figure was created using BioRender.

profile of the endometrial tissue of women with endometriosis at the receptive phase remained suitable for quantitative analysis (Fig. 2). The pooled dataset of differentially expressed genes composed in total of 125 samples, 78 eutopic endometrial samples from women with endometriosis and 47 endometrial samples from women without endometriosis, which served as the control group (Table I). After removal of the duplicated genes within studies, a total number of 7779 genes, 3496 up-regulated and 4283 down-regulated genes, were obtained for further analysis.

The integration of the lists of dysregulated genes using the RRA method led us to detect 74 genes from the 7779 meta-analysed genes showing significant RRA score (<0.05) (Table II). However, none of these genes remained under the significance threshold when the FDR multiple correction was applied. The complete results of the meta-analysis including RRA score and FDR values for each gene are presented in Supplementary Table SI.

The enrichment analysis highlighted biological processes such as chemotaxis and locomotion as significantly enriched simultaneously across all the studies, suggesting a possible dysregulation in endometriosis. For the rest of the search categories, no significant differences between the study groups were detected (Supplementary Table SII).

Endometrial receptivity-specific genes

The dataset of endometrial receptivity genes was prepared for further analysis. In particular, for the ERA test, the 238 genes were ranked according to their absolute value of FC; the 25 ER Map®/ER Grade® test genes were ranked according to their normalized importance; and finally, for the beREADY® test, the 57 genes were ranked according to their RRA score. The full set of selected genes is presented in Supplementary Table SIII.

The intersection among the panel of endometrial receptivity genes and the differentially expressed genes from the dataset from our meta-analysis was calculated. This new subset was subjected to RRA method analysis in order to detect endometrial receptivity biomarkers that could be recurrently altered in our study datasets. Four genes (*C4BPA*, *PAEP*, *MAOA* and *DKK1*) were rated as significantly dysregulated in our meta-analysis, while *C4BPA*, *PAEP* and *MAOA* remained significant after FDR correction (Supplementary Table SIV). Next, we were interested in detecting the underlying functional processes in which these RRA-ranked receptivity-specific transcripts are involved through a functional enrichment analysis, where the intersection analysis of enriched processes between each endometrial receptivity test and our meta-analysed transcripts detected different molecular functions and biological processes mainly related to immune and defence responses (Fig. 3; Supplementary Table SV).

Data mining of candidate genes

The endometrial receptivity-specific genes *C4BPA*, *PAEP* and *MAOA* dysregulated in women with endometriosis were subjected to further analysis in order to contextualize their role in the endometrial processes in endometriosis. The gene–disease and variant–disease

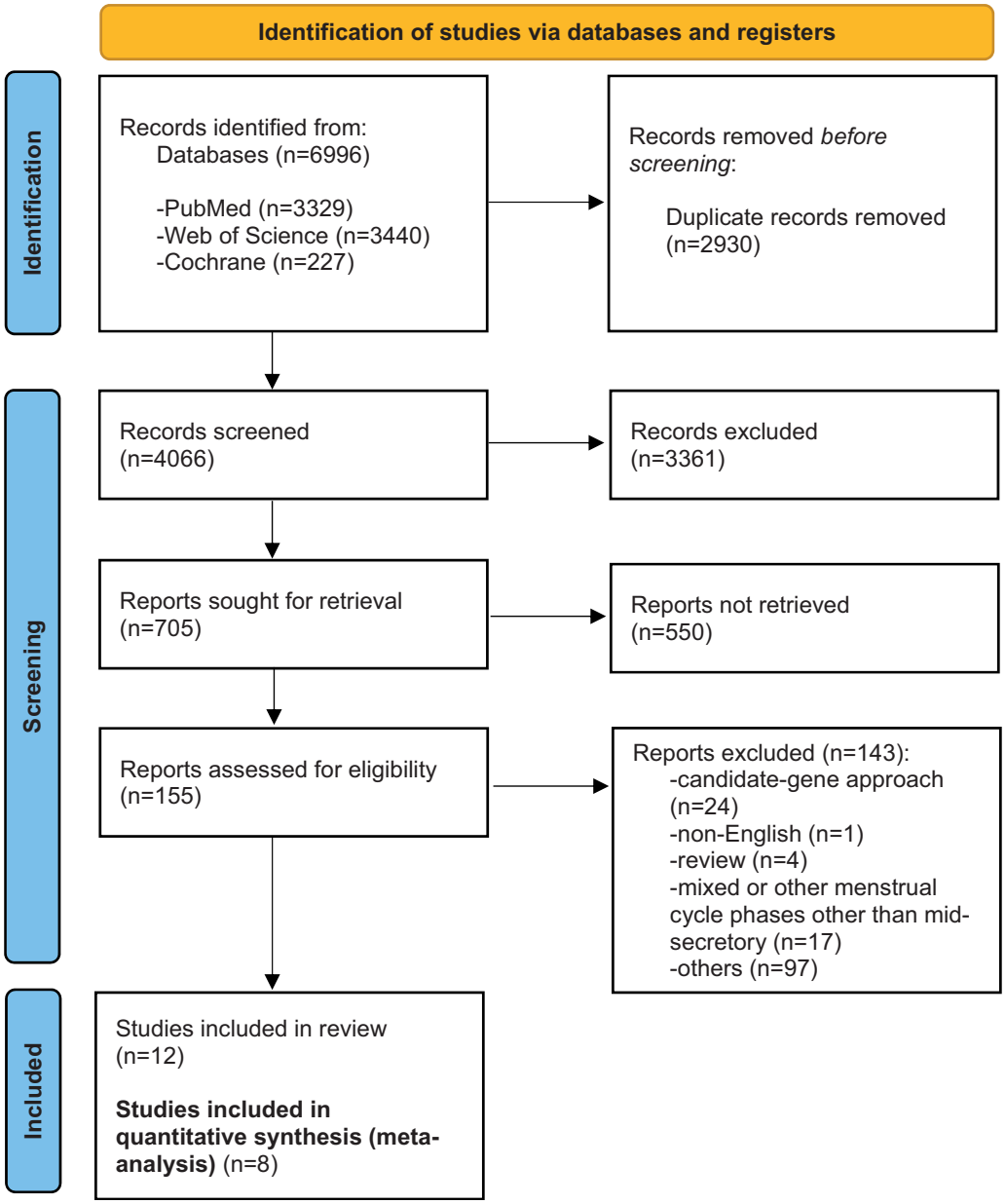

Figure 2. Flow chart of the systematic literature search. Chart depicting the flow of information throughout the phases of the systematic review conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. After exclusion of non-eligible studies, a total number of 12 studies were included in the final list of articles suitable for meta-analysis. Of those, in 4 studies the data were not available, and 8 studies were finally subjected to meta-analysis.

association analyses resulted in 107 diseases and 4 variants in association with the *C4BPA* gene, while 300 diseases and 15 variants were identified for the *MAOA* gene, and 397 diseases and 2 variants were identified for the *PAEP* gene (Supplementary Table SVI). There was an overlap of 18 diseases among the three genes regarding gene–disease associations, and an association between *PAEP* and *MAOA* genes and endometriosis was identified (Fig. 4). No associations between the variants and disease were detected (Supplementary Table SVI).

Furthermore, a gene interaction network was constructed in Cytoscape, where *C4BPA*, *PAEP* and *MAOA* genes were connected to 20 other molecules (Fig. 5). Of interest are genes such as the progesterone receptor (*PGR*), *DNAJB5*, *PTPRC*, *CD40* and *NFKBIA*, which were also identified in our dataset of meta-analysis, and the *C3*, *CD55* and *NDRG1* genes among the endometrial receptivity-specific biomarker list.

Discussion

Our study presents the first meta-analysis approach in detecting the endometrial receptivity transcriptome in endometriosis and

Table I Studies selected for data extraction for meta-analysis after the systematic literature search.

Authors	Controls/patients with endometriosis (sample size)	Array/sequencing platform	Cycle phase	Signif. threshold	FC threshold	Up-regulated transcripts	Down-regulated transcripts
Ahn *et al.* (2016)	**Controls (n = 8):** Healthy women undergoing tubal ligation. No hormonal therapy for 3 months before sampling. **Patients (n = 8):** Stage III–IV endometriosis with infertility and/or pelvic pain. No hormonal therapy for 3 months before sampling.	nCounter human Immunology v2 panel	Sec.*	$P \leq 0.05$	>1.5	60	31
Burney *et al.* (2007)	**Controls (n = 8):** Normally cycling women, volunteers to donate sample, no endometrial inflammation. No hormonal treatment for 3 months before sampling. **Patients (n = 9):** Surgically documented and histologically validated moderate/severe-stage endometriosis. No hormonal treatment in preceeding 3 months.	Affymetrix Human U133-Plus 2.0	MS	FDR <0.05	>1.5	428	293
Kao *et al.* (2003)	**Controls (n = 7):** Normally cycling women. Surgically confirmed without endometriosis. No medication. **Patients (n = 8):** Surgically documented mild/moderate endometriosis. No medication.	Affymetrix Genechip Hu95A	MS	$P < 0.05$	>2	63	86
Matsuzaki *et al.* (2005)	**Controls (n = 3):** Fertile women with regular cycles undergoing tubal ligation or sterilization. No hormonal treatment in preceeding 6 months. **Patients (n = 3):** Surgically confirmed deep endometriosis. No hormonal treatment for 6 months before surgery.	Clontech Atlashuman 1.2 cDNA expression array	MS	$P < 0.05$	>3	6**	20**
Tamaresis *et al.* (2014)	**Controls (n = 8):** No uterine/pelvic pathology, healthy volunteers for tubal ligation or endometrial biopsy. No hormonal treatment in preceeding 3 months. **Patients (n = 28):** Laparoscopically confirmed endometriosis and pelvic pain and/or infertility. No hormonal treatment in preceeding 3 months.	Affymetrix Human U133-Plus 2.0	MS	No threshold	>1.5	4020	6438
Zhao *et al.* (2017)	**Controls (n = 5):** Laparoscopic surgery for examination or hydrotubation. No hormonal therapy. **Patients (n = 8):** Endometriosis confirmed by histology, stage III–IV. No hormonal therapy.	Illumina HiSeq4000 system	Sec.*	FDR ≤0.05	≥2 (up) ≤0.5 (down)	66	6
Da Broi *et al.* (2019)	**Controls (n = 5):** Fertile healthy women with regular cycles, undergoing tubal ligation. No medication. **Patients (n = 6):** Infertility exclusively associated to endometriosis (diagnosed by laparoscopy). No medication.	Illumina HiSeq2500 System	MS	adj $P < 0.05$	log2 > 1 log2 < 1	0	0
Joshi *et al.* (2021)	**Controls (n = 3):** Healthy endometriosis-free women. Regular cycles. No hormonal therapy for 3 months before sampling. **Patients (n = 8):** Endometriosis confirmed with laparoscopy and pathology. No hormonal therapy in preceding 3 months.	Affymetrix Human Gene 1.0 ST Arrays	MS	FDR ≤0.05	>1.5	0	0
	Total number of samples: Controls n = 47; Endometriosis n = 78	**Total number of differentially expressed genes (with duplicates)**				**4643**	**6874**
		Total differentially expressed genes (with real symbol and FC)				**4286**	**6465**
		Total number of unique differentially expressed genes				**3496**	**4283**

adj *P*: adjusted *P*-value; FC, fold change; FDR, false discovery rate; MS, mid-secretory phase; Sec.: secretory phase.
*Secretory phase not specified but the focus on the study was on receptive phase.
**Gene lists from epithelial and stromal cells were merged.

Table II List of meta-analysed dysregulated genes after application of Robust Rank Aggregation (RRA) method.

Gene symbol	Gene name	RRA score	FDR
FOSB ↑	FosB Proto-Oncogene, AP-1 Transcription Factor Subunit	4.12E−05	0.304
S100A8 ↑	S100 Calcium Binding Protein A8	0.00013341	0.492
FOS ↑	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit	0.00086691	1
GUCY1B2 ↑↓	Guanylate Cyclase 1 Soluble Subunit Beta 2 (Pseudogene)	0.00157031	1
CFB ↑	Complement Factor B	0.00211388	1
IL32 ↑	Interleukin 32	0.00247818	1
SELL ↑↓	Selectin L	0.00329218	1
CD4 ↑↓	CD4 Molecule	0.00421945	1
EGR1 ↑	Early Growth Response 1	0.00455357	1
CYR61 ↑	Cysteine-rich angiogenic inducer 61	0.00472531	1
PPBP ↑	Pro-Platelet Basic Protein	0.0048764	1
CASP5 ↑	Caspase 5	0.0048764	1
WT1 ↓	WT1 Transcription Factor	0.0048764	1
RP11-319E12.2 ↑	Clone-based (Vega) gene	0.0048764	1
SOCS1 ↑	Suppressor of cytokine signalling 1	0.00724011	1
IL17A ↑	Interleukin 17A	0.00974949	1
LTB4R2 ↑	Leukotriene B4 Receptor 2	0.00974949	1
htMART ↑	Putative mono-ADP-ribosyltransferase	0.00974949	1
ATP7B ↓	ATPase Copper Transporting Beta	0.00974949	1
IGFBP1 ↑	Insulin Like Growth Factor Binding Protein 1	0.00974949	1
NR4A1 ↑	Nuclear Receptor Subfamily 4 Group A Member 1	0.01027371	1
CTNNB1 ↑↓	Catenin Beta 1	0.01029457	1
C4BPA ↑↓	Complement Component 4 Binding Protein Alpha	0.01211943	1
HES1 ↑	Hes Family BHLH Transcription Factor 1	0.01440884	1
CXCL10 ↑	C-X-C Motif Chemokine Ligand 10	0.01461928	1
PTAFR ↑	Platelet Activating Factor Receptor	0.01461928	1
NAB50 ↑	RNA-binding protein CUG-BP/hNab50	0.01461928	1
SLC1A1 ↓	Solute Carrier Family 1 Member 1	0.01461928	1
LRRC26 ↓	Leucine Rich Repeat Containing 26	0.01461928	1
LTBR ↑↓	Lymphotoxin Beta Receptor	0.01819934	1
IL7R ↑	Interleukin 7 Receptor	0.01948577	1
VSTM2L ↑	V-Set and Transmembrane Domain Containing 2 Like	0.01948577	1
BSEP ↑	Bile Salt Export Pump	0.01948577	1
SOS1 ↑↓	SOS Ras/Rac Guanine Nucleotide Exchange Factor 1	0.01948577	1
C1orf63 ↓	Arginine And Serine Rich Protein 1	0.01948577	1
SERPINB2 ↑	Serpin Family B Member 2	0.01948577	1
CD19 ↑	CD19 Molecule	0.02434897	1
ASB2 ↑	Ankyrin Repeat and SOCS Box Containing 2	0.02434897	1
VDAC1P1 ↑	Voltage Dependent Anion Channel 1 Pseudogene 1	0.02434897	1
SMG1 ↓	SMG1 Nonsense Mediated MRNA Decay Associated PI3K Related Kinase	0.02434897	1
CFH ↑	Complement Factor H	0.02920887	1
GZMA ↑	Granzyme A	0.02920887	1
NEAT1 ↑↓	Nuclear Paraspeckle Assembly Transcript 1	0.02920887	1
ZIC2 ↑	Zic Family Member 2	0.02920887	1
SLC6A7 ↓	Solute Carrier Family 6 Member 7	0.02920887	1
EREG ↑	Epiregulin	0.02920887	1
MPPED2 ↑↓	Metallophosphoesterase Domain Containing 2	0.02928315	1
ANK3 ↑↓	Ankyrin 3	0.03015014	1
IER3 ↑↓	Immediate Early Response 3	0.03035717	1

(continued)

Table II Continued

Gene symbol	Gene name	RRA score	FDR
CXCR2 ↑	C-X-C Motif Chemokine Receptor 2	0.03406547	1
LOC389332 ↑	Small Integral Membrane Protein 32	0.03406547	1
TRPM6 ↓	Transient Receptor Potential Cation Channel Subfamily M Member 6	0.03406547	1
CA1 ↑	Carbonic Anhydrase 1	0.03406547	1
ITGAL ↑	Integrin Subunit Alpha L	0.03406547	1
MMP27 ↑	Matrix Metallopeptidase 27	0.03406547	1
HTRA3 ↑	HtrA Serine Peptidase 3	0.03521064	1
WAS ↑↓	WASP Actin Nucleation Promoting Factor	0.0375287	1
CXCL9 ↑	C-X-C Motif Chemokine Ligand 9	0.03891879	1
SAP30L ↑↓	SAP30 Like	0.03891879	1
PMS7 ↑	HPMS7 protein	0.03891879	1
PTPN11 ↓	Protein Tyrosine Phosphatase Non-Receptor Type 11	0.03891879	1
ABP1 ↑	Actin-binding protein	0.03891879	1
S100A7 ↓	S100 Calcium Binding protein A7	0.03891879	1
SAMD14 ↑	Sterile Alpha Motif Domain Containing 14	0.04376881	1
PRIM2 ↑	DNA primase large subunit	0.04376881	1
DYNLL1 ↑	Dynein Light Chain LC8-Type 1	0.04376881	1
CTSW ↑	Cathepsin W	0.04376881	1
MMP10 ↑	Matrix Metallopeptidase 10	0.04376881	1
KLRG2 ↑	Killer Cell Lectin Like Receptor G2	0.04861554	1
ORAI2 ↑	ORAI Calcium Release-Activated Calcium Modulator 2	0.04861554	1
IFNA21 ↑	Interferon Alpha 21	0.04861554	1
MAP3K8 ↓	Mitogen-Activated Protein Kinase Kinase Kinase 8	0.04861554	1
CCL3 ↑	C-C Motif Chemokine Ligand 3	0.04861554	1
LEFTY2 ↑	Left-Right Determination Factor 2	0.04861554	1

For each gene, corresponding RRA score and false discovery rate (FDR) multiple correction values are presented. Arrows indicate the up- (↑) and down-regulated (↓) expression of the transcripts in the original datasets. Please note that for some genes, both up- and down-regulated expression can be observed among different studies (↑↓).

demonstrates molecular differences among women with endometriosis when compared with women without the disease at the receptive phase endometria, although the molecular differences were small-scale.

There is an ongoing debate about whether the endometrial implantation potential of women with endometriosis is impaired or not (Garcia-Velasco *et al.*, 2015; Lessey and Kim, 2017; Miravet-Valenciano *et al.*, 2017). The possibility of the altered endometrial receptivity in eutopic endometria of women with endometriosis is based on the fact that endometriosis impacts cycle fecundity through the systemic and local inflammatory changes that take place as a consequence of the disease (Lessey and Kim, 2017). In a previous study of 240 IVF cycles, where sibling oocytes from the same donor were transferred into women with endometriosis and without the disease, reduced implantation and pregnancy rates were demonstrated among the endometriosis group (Prapas *et al.*, 2012). Furthermore, a recent matched cohort study on 1053 IVF/ICSI cycles with fresh single embryo transfers demonstrated that in women with endometriosis an impaired implantation factor contributed to the reduced pregnancy outcomes (Blank *et al.*, 2021). On the other side, many studies support the clinical observations that eutopic endometrium is not impaired in women with endometriosis, based on the results of IVF and oocyte donation programs (Miravet-Valenciano *et al.*, 2017; Saare *et al.*, 2017; Da Broi *et al.*, 2019). With the first meta-analysis approach focusing on receptive phase endometria where we analysed carefully selected studies of endometrial transcriptome profiles in 125 women, molecular differences in women with endometriosis were detected, although the differences were minimal.

Biological processes such as chemotaxis and locomotion were dysregulated in the endometria of women with endometriosis. These processes have been previously connected to endometriosis (Devesa-Peiro *et al.*, 2020). Locomotion encompasses a variety of processes involving the self-propelled movement of a cell from one location to another. In endometriosis, cell migration processes of the endometrial tissue are considered an important factor to explain the pathogenesis of the disease (Saare *et al.*, 2017). Indeed, it has been described that aberrant cell migration patterns might result in the impairment of the function of endometrial and endometriotic epithelial and stromal cells of women with endometriosis (Matsuzaki and Darcha, 2013). Altered endometrial cellular composition and functionality in endometriosis has recently been demonstrated (Bunis *et al.*, 2022). In line with these findings, a recent *in silico* analysis of transcriptome studies has proposed

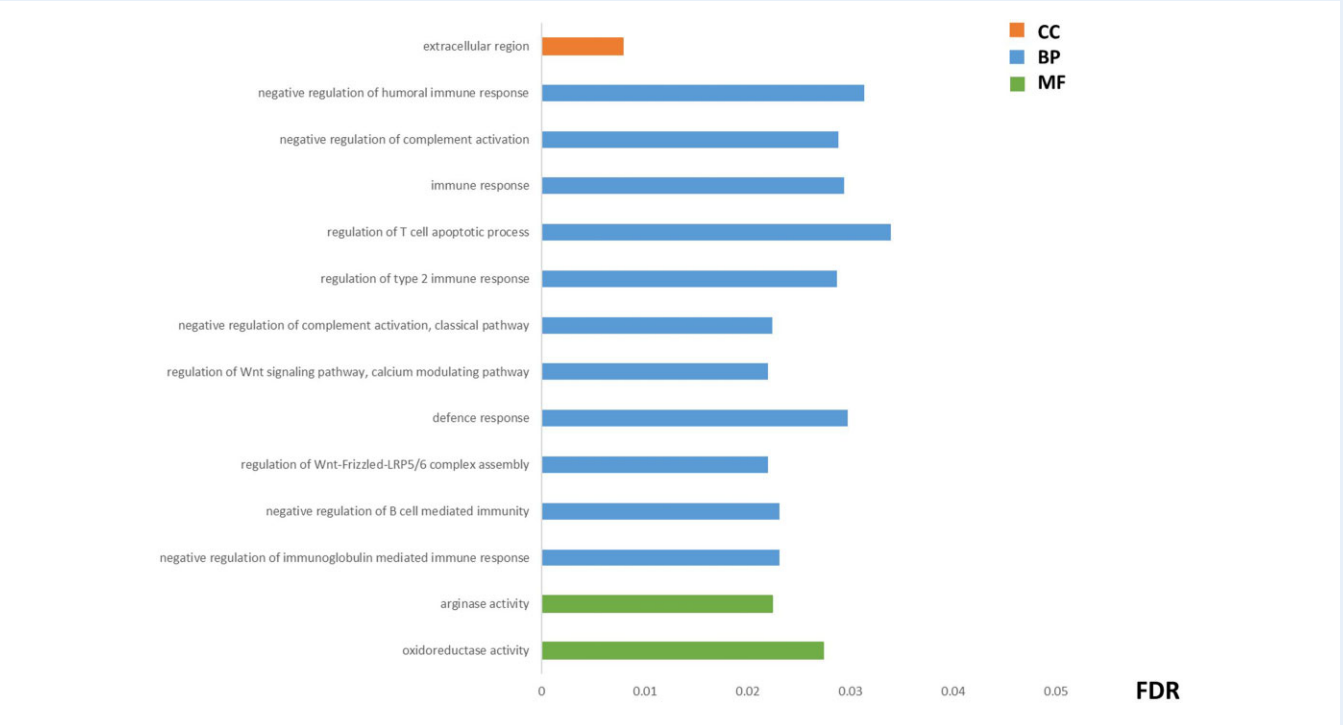

Figure 3. Functional enrichment analysis of the endometrial receptivity genes within the meta-analysed transcripts. The most representative items detected were consistently dysregulated in the intersection between each endometrial receptivity test and the studies included in the meta-analysis (endometrial receptivity array (ERA) versus studies; ER Map®/ER Grade® test versus studies; and beREADY® test versus studies). Only the processes that have a significant false discovery rate (FDR) across the three comparisons are shown. The values of FDR in the figure correspond to the average value of the FDR for each comparison. Complete details of the results of the meta-analysis are described in Supplementary Table SV. BP, biological processes; CC, cellular components; MF, molecular functions.

the involvement of 22 potential biomarkers involved in cell motility and migration in both the ectopic and eutopic endometria of women with endometriosis (Devesa-Peiro *et al.*, 2021). Furthermore, chemotaxis was perturbed in women with endometriosis due to the action exerted by oestrogens and progestogens, which would contribute to the known hallmarks of endometriosis, such as inflammatory response or abnormal tissue remodelling, among others (Reis *et al.*, 2013). Also in previous endometrial transcriptome studies in women with endometriosis slight gene expression differences or no differences have been detected (Garcia-Velasco *et al.*, 2015; Ahn *et al.*, 2016; Miravet-Valenciano *et al.*, 2017; Saare *et al.*, 2017; Da Broi *et al.*, 2019; Joshi *et al.*, 2021). With our meta-analysis approach, we were able to increase the sample size and thereby the power in detecting small molecular differences which could have been lacking in some of the previously published studies (Thorlund and Mills, 2012; Serdar *et al.*, 2021). Altogether, the complex dynamics of the endometrial tissue could underlie the little variations in gene expression and could explain the fact that the affected biological processes and molecular pathways tend to have a common root, although the individual gene expression patterns show low overlap among the studies involved (McKinnon *et al.*, 2018).

Next, our analysis focusing on the endometrial receptivity-specific genes highlighted the dysregulation of three known biomarkers of endometrial receptivity *C4BPA*, *MAOA* and *PAEP* genes in the endometria of women with endometriosis. Abnormally decreased levels of *C4BPA* (Complement Component 4 Binding Protein Alpha) have been detected in the mid-secretory endometrium of women with endometriosis, implantation failure and unexplained recurrent abortion (Kao *et al.*, 2003; Tapia *et al.*, 2008; Herington *et al.*, 2016; Altmäe *et al.*, 2017), suggesting its possible influence in the mechanisms leading to successful embryo implantation. C4BPA has also been identified as candidate target marker in ovarian clear cell carcinomas (Mikami *et al.*, 2015), a well-known comorbidity of endometriosis (Vargas *et al.*, 2020). *MAOA* (Monoamine Oxidase A) is a putative endometrial receptivity biomarker (Altmäe *et al.*, 2017; Wang *et al.*, 2020), which was further linked to endometriosis in our *in silico* data mining according to the DisGeNET analysis.

PAEP (Progestagen-Associated Endometrial Protein), also named as glycodelin, is a protein with immunosuppressive properties and has been described to take part in the endometrial receptivity processes (Oehninger *et al.*, 1995; Seppälä *et al.*, 1998; Kao *et al.*, 2003; Tapia *et al.*, 2008; Vargas *et al.*, 2012; Herington *et al.*, 2016; Altmäe *et al.*, 2017; Wang *et al.*, 2020) and in different aspects of endometriosis (O *et al.*, 2018). Interestingly, its implication in endometriosis-related infertility and impaired receptivity has been proposed (Dutta *et al.*, 2001; Focarelli *et al.*, 2018). PAEP protein is expressed in the endometrial glandular compartment among patients with endometriosis (Kämäräinen *et al.*, 1993). Furthermore, direct correlation of plasma concentrations of PAEP with the severity of deep infiltrating endometriosis has been reported (Koninckx *et al.*, 1992). More recently, PAEP

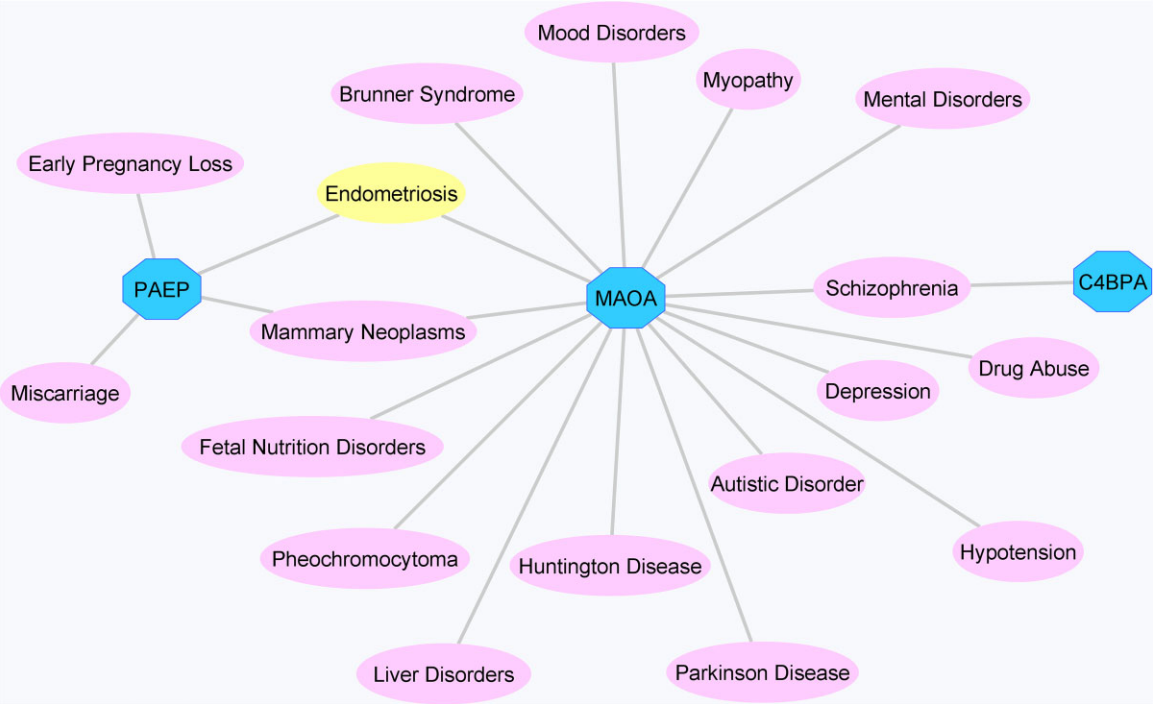

Figure 4. Gene–disease association network for the *C4BPA*, *MAOA* and *PAEP* genes created using the information displayed by DisGeNET plugin implemented in Cytoscape software. Genes are represented by octagons, while associated diseases are shown as ellipses.

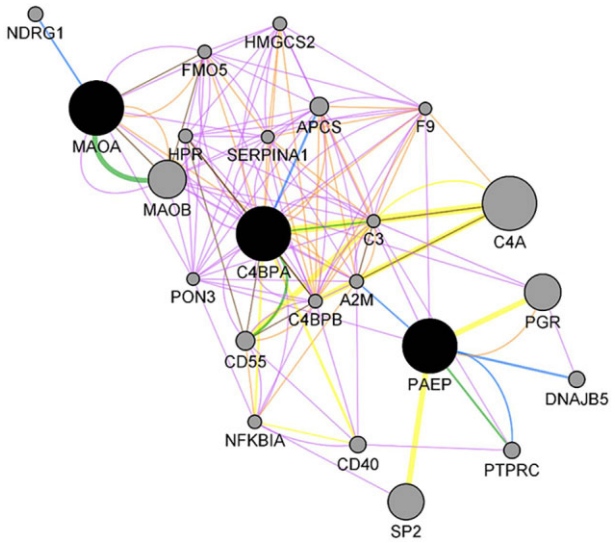

Figure 5. Gene network analysis showing the interaction among the most relevant genes (*C4BPA*, *MAOA* and *PAEP*). Seed genes are shown in black, while interactors appear in grey. The colour of the connection denotes the type of interaction established among the different nodes: blue for physical interactions (67.64% of interactions); purple for co-expression (13.50%); green for predicted interactions (6.35%); orange for co-localisation (6.17%); yellow for shared pathways (4.35%); and brown for shared protein domains (0.59%).

concentration has been reported as significantly higher in the peritoneal fluid of patients with endometriosis compared to controls in both the proliferative and secretory phases of the menstrual cycle (Nirgianakis *et al.*, 2020). Furthermore, a recent study using organoids expressing glycodelin demonstrated that those deriving from the eutopic endometrium of women with endometriosis exhibited a glycosylation pattern significantly different from that of organoids from healthy women (Luddi *et al.*, 2020). Our *in silico* data mining detected a number of important genes directly interacting with *PAEP*, *MAOA* and *C4BPA* genes such as *PGR*, *CD40*, *CD55*, *NFKBIA* or *SERPINA1*, suggesting that these biomarkers could be involved in different molecular processes and pathways. Thus, future studies should investigate the role of these endometrial receptivity biomarkers, *PAEP*, *MAOA* and *C4BPA*, in endometrial function and whether they could serve for identifying an impaired implantation factor in women with endometriosis undergoing IVF/ICSI treatments and direct clinical management.

The intersection analysis of the endometrial receptivity-specific transcripts with our meta-analysed data led to identification and confirmation of dysregulation of biological processes relevant to the aetiopathogenesis of endometriosis, such as immune and defence responses. The association between endometriosis and immunity has been largely demonstrated over the years, with reports of abnormalities in the immune system of women with endometriosis that may be a reflection of the inflammatory response developed during the disease (Shigesi *et al.*, 2019; Poli-Neto *et al.*, 2020). Indeed, it is claimed that the presence of endometriosis predisposes to, or is associated with, the development of autoimmune conditions (Kvaskoff *et al.*, 2014; Shigesi *et al.*, 2019; Vargas *et al.*, 2020). In line, our *in silico* data mining detected hundreds of diseases associated with the endometrial receptivity biomarkers dysregulated in our study set of endometriosis.

Some limitations of our meta-analysis should be highlighted. The lack of available raw data and/or full gene lists allowed us to focus only on the differentially expressed gene lists, which could have reduced the sensitivity of the findings. Also, not all women in the control group were with proven fertility, and although most of the volunteers in the control group were surgically confirmed to be endometriosis-free, we cannot rule out that a few of them had endometriosis, thereby possibly minimizing the differences between study groups. Furthermore, the stringent criteria utilized for the selection of the differentially expressed genes after running the meta-analysis does not allow us to exclude the null hypothesis, meaning that we cannot rule out the dysregulation of specific genes in endometrium of women with endometriosis. Studies on bigger sample size and better-defined control groups are warranted.

In conclusion, we were able to compile a long list of differentially expressed genes in different studies through a systematic review. Moreover, when we integrated the sets of genes originating from different studies through a meta-analysis, the functional enrichment analysis detected a slight molecular dysregulation where biological processes such as chemotaxis and locomotion were involved. Regarding the analysis of endometrial receptivity-specific genes, *C4BPA*, *MAOA* and *PAEP* expression and molecular pathways involved in the immune and defence responses were dysregulated among women with endometriosis. In short, our meta-analysis detected slight molecular differences in the transcriptome profile in endometriosis that could explain, at least in part, the impaired reproductive outcomes in some women with endometriosis. Further research of the molecules and pathways identified in biomarker and therapeutical applicability is warranted to make these findings clinically relevant.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

Data availability

All data are incorporated into the article and its online supplementary material.

Authors' roles

S.A. and E.V. conceived the study and performed the systematic review. E.G.-M. and E.V. analysed the data. F.J.E. assisted in the experimental design and data analysis. S.A. and E.V. wrote the main body of the manuscript. A.S., L.A. and J.A.H. gave advice on the writing of the manuscript. All the authors participated actively in the critical revision of the manuscript.

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Conflict of interest

The authors declare no competing interests.

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