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MOLECULAR NETWORK ANALYSIS OF ENDOMETRIOSIS

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Abstract:

Endometriosis is a condition that affects many women, where tissues like the lining of the uterus grow outside the uterus. It can lead to very painful periods, pelvic pain, intercourse pain, and infertility. Though it affects many women across the globe, the causes of endometriosis are still not well understood. However, research has shown that hormonal imbalance, particularly estrogen; immune system dysfunction; chronic inflammation; and genetic predisposition are all associated with endometriosis. In this research, bioinformatics techniques were employed to uncover the key genes and pathways that are associated with endometriosis. A list of genes associated with endometriosis was first compiled, and the interactions among these genes were then examined. Hub genes such as TNF, IL6, VEGFA, ESR1, PTGS2 and MMP9 were identified as central regulators within the interaction network. These genes were found to be mainly involved in inflammation, immune response, estrogen signaling, and angiogenesis. Such pathways help explain how endometriotic tissue survives and grows outside the uterus..

Keywords: *Endometriosis, Bioinformatics Analysis, Hub Genes, Estrogen Signaling, Inflammatory Pathways.*

Introduction

Endometriosis is a common long-term gynecological condition in which tissue similar to the lining of the uterus grows outside the uterine cavity, often affecting the ovaries, pelvic region, and nearby organs (1). It affects nearly 10% of women of reproductive age and is associated with symptoms such as pelvic pain, painful periods, discomfort during intercourse, and infertility. These symptoms can significantly affect physical health, emotional well-being, and overall quality of life. Despite its high prevalence, the exact cause of endometriosis is still not fully understood because the disease develops through multiple interacting factors.

Several theories have been proposed to explain how endometriosis develops. One of the most widely accepted explanations is retrograde menstruation, where menstrual tissue flows backward into the pelvic cavity and implants there. However, since this process also occurs in women without the disease, additional mechanisms such as coelomic metaplasia, stem cell involvement, and impaired immune function are also considered important

(2). Studies show that immune system dysfunction may allow misplaced endometrial cells to survive and grow, while persistent inflammation supports lesion development.

Recent studies highlight the role of genetic and molecular factors in increasing disease risk. Genetic research has shown links between endometriosis and autoimmune disorders, suggesting shared biological pathways (3). Estrogen plays a key role in stimulating tissue growth, while epigenetic changes and oxidative stress influence gene expression and disease progression (4). Additionally, alterations in gut and reproductive tract microbiota may affect immune responses and hormone regulation, contributing to the condition (5, 6).

In this context, bioinformatics and network analysis play an important role in understanding molecular mechanisms. These approaches allow researchers to identify disease-associated genes, study how they interact, and map them to biological pathways. By analyzing gene networks and protein interactions, key regulatory genes and signaling pathways involved in inflammation, immune response, estrogen signaling, and angiogenesis can be identified. Such integrated analysis helps in uncovering complex molecular connections and supports the development of potential biomarkers and targeted therapies for endometriosis.

Methodology

1. Identification of endometriosis-associated genes using DisGeNET

DisGeNET is a comprehensive platform integrating gene–disease associations from curated repositories, GWAS studies, and scientific literature (7). It provides structured information on genes implicated in complex diseases, including gynecological disorders. In this study, endometriosis was searched in the DisGeNET database to obtain a list of genes associated with disease susceptibility and progression. The retrieved gene list was filtered based on association score and relevance. These genes served as the primary dataset for subsequent bioinformatics analyses focusing on inflammatory, hormonal, and immune-related mechanisms known to contribute to endometriosis (8).

2. Protein–protein interaction network analysis using STRING

The STRING database was used to construct protein–protein interaction (PPI) networks. STRING integrates experimental data, computational prediction methods, and public database information to predict functional protein associations (9). The endometriosis-associated gene list obtained from DisGeNET was uploaded into STRING, and interaction networks were generated using high-confidence score filtering. Network analysis enabled identification of functional clusters and hub proteins involved in inflammatory signaling, estrogen receptor pathways, immune cell activation, and angiogenesis, which are central to endometriosis pathogenesis (10).

3. Gene interaction and functional association analysis using GeneMANIA

GeneMANIA is a gene function prediction tool that identifies gene interactions based on co-expression, co-localization, pathway association, and genetic interaction datasets (11). The endometriosis gene list was submitted to GeneMANIA to identify additional functionally related genes. The generated interaction network helped identify genes involved in estrogen signaling, immune modulation, extracellular matrix remodeling, and inflammatory cytokine production—key biological mechanisms implicated in endometriosis development (12).

4. Functional enrichment analysis using enrichr

Enrichr is an interactive enrichment analysis tool that integrates multiple gene set libraries and provides comprehensive functional annotation (Kuleshov et al., 2016). The endometriosis-associated gene list was uploaded into Enrichr to perform Gene Ontology (GO) and pathway enrichment analysis. The enrichment results

highlighted biological processes such as immune response, inflammatory signaling, angiogenesis, oxidative stress response, and hormone-mediated signaling pathways, which are widely reported in endometriosis pathophysiology (12).

5. Pathway mapping using reactome database

Reactome is a curated open-source database providing detailed insights into biological pathways and molecular interactions (13). The endometriosis gene set was uploaded into Reactome pathway analysis to identify significantly enriched pathways. The analysis revealed pathways associated with cytokine signaling, TNF-mediated inflammation, estrogen receptor signaling, and angiogenic processes. These pathways are critically involved in lesion establishment, immune dysregulation, and chronic inflammation observed in endometriosis (10).

Result

1. DisGeNET

To investigate the molecular basis of endometriosis, gene–disease association analysis was performed using the DisGeNET database. DisGeNET integrates curated data from scientific literature, GWAS studies, and expert-reviewed repositories to provide comprehensive information about genes implicated in human diseases. Because endometriosis is a multifactorial and inflammatory disorder, identifying disease-associated genes is a crucial first step toward understanding its molecular mechanisms.

A total of 30 genes were identified as significantly associated with endometriosis. After filtering based on association score and relevance, 30 genes were selected for downstream analysis. These genes are reported to be involved in inflammatory signaling, immune cell activation, hormonal regulation, angiogenesis, and extracellular matrix remodeling. The selected gene set forms the foundational dataset for subsequent interaction network and pathway enrichment analyses.

2. STRING:

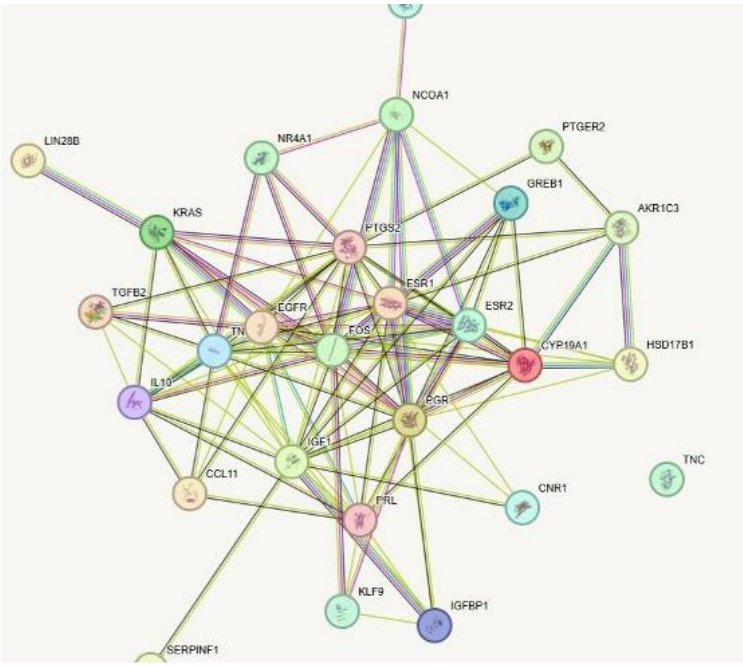

Figure 1: String Analysis of the genes involved in endometriosis

The selected endometriosis-associated genes were uploaded into the STRING database to construct a protein–protein interaction (PPI) network. PPI analysis helps in understanding how proteins function collectively rather than individually, revealing coordinated molecular interactions underlying complex diseases. Since endometriosis involves immune and hormonal dysregulation, network analysis provides insight into interconnected signaling pathways.

The interaction network demonstrated significant functional associations among the selected genes, indicating coordinated regulation of inflammatory and estrogen-dependent signaling pathways. Several highly connected nodes were observed, suggesting the presence of potential hub proteins that may play central roles in lesion formation, immune activation, and chronic inflammation characteristic of endometriosis.

GO biological process table:

Gene Ontology enrichment analysis revealed significant involvement of biological processes such as immune response, cytokine-mediated signaling, inflammatory regulation, cell proliferation, and angiogenesis. These processes are consistent with the known pathophysiological features of endometriosis, where ectopic endometrial tissue survival is supported by immune dysfunction and increased vascularization.

Theme no.	Biological theme	Representing GO Terms
1	Inflammatory Response & Cytokine Signaling	Inflammatory response, cytokine-mediated signaling pathway, regulation of immune response
2	Immune System Modulation	Leukocyte activation, T-cell activation, macrophage differentiation
3	Estrogen & Hormonal Regulation	Response to estrogen, steroid hormone-mediated signaling
4	Angiogenesis & Vascular Development	Blood vessel development, angiogenesis, endothelial cell proliferation
5	Cell Proliferation & Tissue Remodeling	Cell proliferation, extracellular matrix organization, cell adhesion

The thematic grouping of enriched Gene Ontology (GO) terms provides a clearer understanding of the biological mechanisms underlying endometriosis. The predominance of inflammatory response and cytokine signaling pathways highlights the chronic inflammatory nature of the disease, where elevated levels of pro-inflammatory mediators promote lesion survival and pelvic pain. Enrichment of immune modulation processes suggests altered immune surveillance, allowing ectopic endometrial cells to evade immune clearance.

The identification of estrogen-related signaling pathways further confirms the hormone-dependent characteristics of endometriosis, as estrogen stimulates proliferation and sustains lesion growth. Additionally, angiogenesis-associated terms indicate the formation of new blood vessels necessary for the maintenance of ectopic implants. Processes related to cell proliferation and extracellular matrix remodeling reflect the invasive and fibrotic behavior of lesions. Overall, these enriched biological themes collectively demonstrate that endometriosis is driven by interconnected inflammatory, hormonal, immune, and tissue-remodeling mechanisms.

3. GeneMANIA

GeneMANIA was employed to explore gene–gene interactions and functional associations among the identified endometriosis-related genes. This analysis helps predict additional interacting genes and biological functions

based on co-expression, physical interactions, shared pathways, and genetic interactions.By constructing an interaction network, GeneMANIA helps in understanding how genes work collectively within biological systems rather than functioning independently. In complex disorders such as endometriosis, gene interactions play a crucial role in regulating inflammatory responses, hormonal signaling, immune modulation, and tissue remodeling. Therefore, network analysis provides deeper insight into coordinated gene regulation and reveals potential key regulatory genes involved in disease progression.

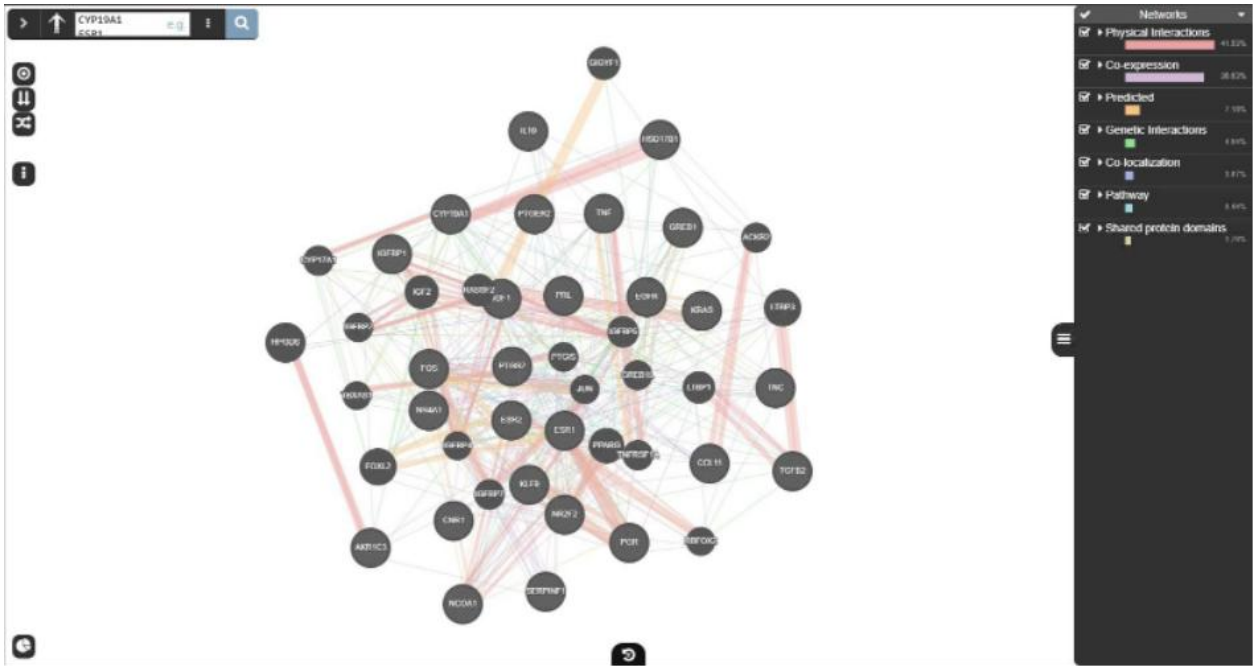

Figure 2: Genemania Analysis for gene-gene interaction

The figure represents a protein-protein interaction network where several highly interconnected genes are emphasized instead of a single hub gene. Although CYP19A1 is a central gene with significant functional importance in hormone synthesis, there are several other genes that show high connectivity and play a crucial role as central regulatory genes in the network. The major highly interconnected genes include ESR1, JUN, FOS, TNF, KRAS, PTGS2, and NFKB genes, which together constitute a dense core of interaction. These genes are primarily involved in transcription regulation, inflammation, cell proliferation, and endocrine regulation. The high connectivity of these genes reflects their coordinated expression and common participation in various biological pathways. The edges of the network denote various types of interactions, including physical interactions, co-expression, pathway membership, and predicted functional associations. The presence of several hub genes in the network reflects the presence of a multi-level regulatory mechanism, where the interaction of hormonal regulation (CYP19A1, ESR1), inflammatory mediators (TNF, PTGS2, NFKB), and transcription factors (JUN, FOS) regulates cellular responses

4. Enrichr

To further characterize the biological significance of the selected gene set, enrichment analysis was performed using Enrichr. This tool integrates multiple gene set libraries to identify overrepresented biological functions and signaling pathways. Enrichment results demonstrated significant association of the gene set with immune response, inflammatory signaling pathways, oxidative stress response, angiogenesis, and hormone-mediated

cellular processes. These findings align with current evidence suggesting that endometriosis is a chronic inflammatory condition influenced by estrogen-dependent mechanisms. The Enrichr enrichment analysis provided deeper insight into the functional characteristics of the selected gene set by evaluating their distribution across multiple gene libraries. Significant enrichment was observed in biological pathways related to inflammatory signaling, immune activation, and hormone-mediated processes, reinforcing the known molecular basis of endometriosis.

Libraries table

The enrichment analysis across multiple gene libraries confirmed the biological relevance of the selected genes in endometriosis. Consistent enrichment across diverse databases strengthens the reliability of the findings and indicates that these genes participate in interconnected regulatory networks involved in inflammation and hormonal signaling.

Table 1: The table summarizes functional annotation of selected genes across multiple biological databases (KEGG, Reactome, WikiPathways, DisGeNET, OMIM, Jensen Disease, ChEA, ENCODE, ARCHS4, and Allen Brain Atlas) and highlights the number of resources supporting each gene.

Gene	KEGG 2021 human	Reactome pathway 2024	Wikipathway 2024 human	DisGeNET	OMIM disease	Jensen disease	ChEA 2022	ENCODE	ARCHS4 human tissues	Allen brain atlas	Count
CYP19A1	YES	YES	YES	YES		YES			YES		6
ESR1	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	10
PGR	YES		YES	YES		YES	YES		YES	YES	7
IGF1	YES	YES	YES	YES	YES	YES	YES	YES	YES		9
KRAS	YES	YES	YES	YES		YES		YES			6
GREB1						YES	YES	YES	YES	YES	4
TNF	YES		YES	YES	YES	YES					5
IGFBP1				YES		YES	YES	YES	YES		5
IL10	YES		YES	YES	YES	YES	YES	YES			7
PRL	YES		YES	YES		YES	YES	YES	YES		7
IL1B											0
PTGS2	YES	YES	YES	YES		YES	YES		YES	YES	8
EGFR	YES	YES	YES	YES	YES	YES	YES	YES	YES		9
CCL11	YES		YES		YES	YES			YES		5
MIR21	YES			YES			YES				3

HSD17B1	YES	YES	YES	YES				YES	YES		6
SERPINF1							YES		YES		2
AKR1C3	YES	YES	YES	YES					YES	YES	6
PTGER2			YES		YES		YES				3
FOS	YES	YES	YES	YES		YES	YES	YES	YES	YES	9
NCOA1	YES	YES	YES	YES							4
NR4A1			YES						YES	YES	3
TNC			YES			YES	YES	YES			4
KLF9			YES			YES	YES	YES	YES		5
NR2F2		YES	YES			YES	YES	YES	YES		6
CNR1						YES		YES			2

Hub Gene Identification

Several genes show strong multi-database support, indicating high biological relevance. ESR1 shows the highest association (Count = 10), followed by IGF1, EGFR, and FOS (Count = 9), suggesting their central roles in signaling, growth regulation, and transcriptional control. PTGS2, IL10, PRL, and PGR also demonstrate broad functional involvement across pathways and disease annotations. Hormone and endocrine-related genes such as CYP19A1, ESR1, PGR, HSD17B1, and NCOA1 are consistently represented, supporting their importance in steroid signaling and reproductive biology. Growth and signaling regulators including KRAS, IGF1, EGFR, and NR2F2 indicate active participation in proliferation and pathway regulation. Immune and inflammatory mediators such as TNF, IL10, IL1B, CCL11, and PTGS2 reflect involvement in immune response and inflammation. Transcriptional regulators (FOS, KLF9, NR4A1) and metabolic genes (AKR1C3, SERPINF1) further contribute to system-level regulation.

5. Reactome

Reactome pathway analysis was conducted to systematically map the selected genes to curated biological pathways. This approach provides a comprehensive overview of molecular cascades involved in disease mechanisms.Reactome pathway analysis was performed to identify significantly enriched biological pathways associated with the selected endometriosis-related genes. Reactome is a curated pathway database that provides detailed insights into molecular interactions, signaling cascades, and biological processes. By mapping the identified genes onto known human pathways, this analysis helps in understanding how these genes collectively contribute to disease mechanisms. The objective of performing Reactome analysis was to determine whether the selected gene set is functionally involved in immune regulation, inflammatory signaling, hormonal response pathways, and tissue remodeling processes that are central to endometriosis pathogenesis.

The visualization generated by Reactome highlights interconnected signaling cascades, offering a clearer understanding of disease-associated molecular mechanisms.

The Reactome analysis identifies significant enrichment of pathways related to hormone signaling, intracellular signaling cascades, and disease mechanisms. Prominent pathways include ESR-mediated signaling, interleukin signaling, nuclear receptor pathways, and signaling by growth factor receptors. These pathways are strongly

linked to cellular communication, immune modulation, and transcriptional regulation. The visualization shows interconnected biological processes such as cell cycle control, DNA repair, gene expression, metabolism, and programmed cell death, indicating system-level involvement of the gene set. High pathway enrichment scores suggest coordinated activation of signaling networks associated with proliferation, inflammation, and endocrine regulation.

Figure 3: Reactome pathway overrepresentation analysis highlighting enriched signaling and disease-related pathways

Conclusion

This research work offers a holistic systems-level perspective on endometriosis via integrated bioinformatics and network analysis. Gene-to-disease association, protein interaction mapping, functional enrichment, and pathway analysis, taken together, clearly show that endometriosis is a complex condition driven by a network of inflammatory, hormonal, immune, and angiogenic pathways. The detection of 30 disease-causing genes with high interaction scores emphasizes the molecular regulation of disease onset and progression. Network analysis showed the presence of several hub genes, including ESR1, IGF1, EGFR, FOS, TNF, PTGS2, and KRAS, which play pivotal roles in estrogen signaling, immune response, inflammation, and cell proliferation. These genes constitute a highly interconnected regulatory module that might play a crucial role in the survival of lesions, immune evasion, and tissue remodeling.

Gene Ontology and enrichment analysis further supported the significant role of cytokine signaling, immune modulation, estrogen-mediated processes, angiogenesis, and extracellular matrix organization. Reactome pathway analysis further validated these observations by highlighting the significant role of signaling pathways involved in nuclear receptor signaling, interleukin pathways, growth factor signaling, and transcriptional regulation. The validation of observations by multiple databases and analysis tools further supports the robustness of the observations and the complex etiology of endometriosis.

In conclusion, the observations made in this study clearly indicate that endometriosis is a complex disease that cannot be explained by a single molecular phenomenon but is rather a result of complex interactions of multiple

pathways, including hormonal imbalances, chronic inflammation, immune system dysfunction, and tissue remodeling. The hub genes and pathways identified in this study may have the potential to be used as biomarkers for early detection and therapeutic targets for the treatment of endometriosis. Although the results are highly informative at the molecular level, they are predictive in nature and need to be verified by experimental studies. Future research should aim at functional analysis of the hub genes that have been identified and their interactions within pathways by using clinical samples and in vitro and in vivo models. Such approaches could help in developing better diagnostic tools and targeted therapies for the management of endometriosis.

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