Integrative Bioinformatics Analysis Of Menopause-Associated Pain And Potential Therapeutic Targets

Sarayu Pratap Kamath[1], Shreosee Ghosh[2], Rupak Roy[3]
[1]*Benenden School, United Kingdom*
[2,3]*SHRM Biotechnologies Pvt. Ltd., Kolkata, India*

***Abstract*—This study aimed to identify genes linking menopause-related molecular changes with pain and assess their potential as therapeutic targets. Gene-expression analysis of GSE12517 compared 11 premenopausal women with 12 untreated postmenopausal women. Targeted literature reviews, protein-association network analysis, pathway enrichment, and evaluation of regulatory and drug-target evidence identified four candidates: ESR1, ESR2, COMT and CYP19A1. None were significantly differentially expressed after multiple-testing correction, and all had equal network centrality rankings. Enriched pathways involved steroid hormone biosynthesis and estrogen signaling. ESR1 and ESR2 linked estrogen signaling with pain-related mechanisms, CYP19A1 contributed to estrogen biosynthesis, and COMT connected catecholamine metabolism with pain modulation. ESR1, ESR2 and COMT warrant further investigation as potential therapeutic targets, while CYP19A1 requires caution because inhibiting estrogen synthesis may worsen musculoskeletal symptoms.**

***Index Terms*—Menopause; pain; estrogen signaling; gene expression; bioinformatics; therapeutic targets.**

I. INTRODUCTION

Muscle and joint pain are commonly reported during the menopausal transition. Changes in estrogen levels may contribute to these symptoms by affecting musculoskeletal tissues, inflammation, and pain processing. However, menopause occurs alongside aging and other changes that can also influence pain, contributing to hormonal changes that are difficult to isolate. Understanding the underlying molecular processes may help clarify these relationships and guide research into potential treatments.

Estrogen acts beyond the reproductive system. It influences immune activity and the pathways the nervous system uses to detect and regulate pain. Its effects are not uniform: estrogen can increase or decrease pain sensitivity depending on the receptor involved, the tissue, and the biological context. Menopause-associated pain therefore cannot be explained simply as a direct consequence of lower estrogen levels. It may instead reflect changes across interacting hormonal, inflammatory and nervous-system pathways.

Genes involved in estrogen production, estrogen signaling and pain modulation provide a useful starting point for examining these connections. However, evidence linking a gene to menopause or pain does not necessarily mean that changing its activity will provide pain relief. Identifying potential therapeutic targets requires consideration of both biological function and the possible consequences of intervention.

This study combined public gene-expression analysis with a targeted literature review to identify genes associated with menopause-related molecular changes and pain. Protein-association networks, pathway enrichment, regulatory evidence, and drug-target information were then assessed to identify biologically plausible candidates for further experimental investigation.

II. MATERIALS AND METHODS

2.1 Data sources and study design

Gene-expression data were obtained from the Gene Expression Omnibus (GEO). We selected GSE12517 as the primary dataset for differential-expression analysis because it profiles gene expression in human peripheral blood leukocytes from women across distinct estrogen-related physiological and treatment states. [1] The dataset included 11 premenopausal women, 12 untreated postmenopausal women, 10

postmenopausal women receiving aromatase inhibitors, and 12 women receiving tamoxifen. [1]

For the primary analysis, premenopausal and untreated postmenopausal samples were retained. Aromatase inhibitor-treated and tamoxifen-treated samples were excluded because both interventions alter estrogen signaling and could confound expression differences attributable to natural menopause-associated estrogen deficiency.

Two additional GEO datasets were reviewed to support construction of the menopause- and pain-associated gene lists. We reviewed GSE56814, which profiles circulating monocytes from premenopausal and postmenopausal women, as supporting human evidence for menopause-associated gene expression. [2] GSE124272 and GSE150408, which profile peripheral blood from individuals with neuropathic pain and healthy controls, were reviewed as supporting human evidence for neuropathic pain-associated gene expression. [3,4] These datasets were used to inform gene-list construction and biological interpretation; they were not subjected to independent differential-expression analysis in the present study.

Because publicly available GEO datasets did not consistently provide comparable adjusted p-values, log2 fold-change values, and sample metadata across menopause and pain conditions, a targeted narrative literature review was used to identify additional menopause-associated and pain-associated genes.

2.2 Identification of menopause-associated genes

Differential gene expression between premenopausal and untreated postmenopausal women in GSE12517 was assessed using GEO2R, accessed on 3 October 2026. GEO2R is a web-based tool for differential-expression analysis of GEO datasets and uses R packages including GEOquery and limma. [5,6] The premenopausal group was defined as the reference group and the untreated postmenopausal group as the comparison group.

Genes with a Benjamini–Hochberg-adjusted p-value of less than 0.05 were considered significantly differentially expressed. For each significant gene, the log2 fold change and adjusted p-value were recorded. Genes were classified as upregulated or downregulated in postmenopausal samples relative to premenopausal samples according to the direction of the log2 fold change. These expression results were combined with menopause-associated genes identified through a targeted literature review to construct a consolidated menopause-associated gene list. PubMed, Google Scholar and NCBI were searched using terms including ‘menopause,’ ‘postmenopausal,’ ‘estrogen signaling,’ ‘estrogen deficiency,’ ‘gene expression,’ ‘inflammatory pathways’ and ‘pain.’ Seventy-five menopause-associated genes were initially identified from 17 screened publications. Genes were retained only where the source study provided direct human or experimental evidence linking the gene to menopause, estrogen signaling, inflammatory signaling or pain-related biological processes.

2.3 Construction of the pain-associated gene list

A pain-associated gene list was compiled from peer-reviewed genetic, genomic and mechanistic studies of musculoskeletal pain, neuropathic pain, nociception, pain modulation, neuroinflammation and estrogen-mediated pain signaling. PubMed, Google Scholar and NCBI were searched using terms including ‘estrogen signaling,’ ‘neuropathic pain,’ ‘inflammatory pathways,’ ‘pain modulation,’ ‘nociception’ and ‘chronic pain.’ Sixty-two pain-associated genes were initially identified from 17 screened publications. The primary sources included systematic reviews of human pain-genetic associations and mechanistic studies of estrogenic regulation of inflammatory pain pathways. [7,8,9,10,11]

Genes were included when the source study reported a direct association with pain sensitivity, nociceptive signaling, monoaminergic pain modulation, inflammatory cytokine signaling, neuroimmune mechanisms, or estrogen-dependent regulation of nociceptive pathways. Genes supported only by unvalidated computational predictions were not included. For each included gene, the pain-related mechanism, evidence type, and source publication were recorded. Genes were categorized according to whether the supporting evidence was genetic, transcriptional, functional, or mechanistic. This list was used as the comparator for identifying genes shared between menopause-associated and pain-associated biological processes.

2.4 Selection of candidate menopause–pain genes

The menopause-associated and pain-associated gene lists were intersected to identify genes common to both categories. Common genes were then assessed

for biological relevance to estrogen signaling, neuroinflammation, or both. We retained genes as candidate menopause-associated pain genes if the literature supported their involvement in estrogen synthesis, estrogen receptor signaling, estrogen metabolism, neuroinflammatory signaling, or pain processing. Of the common genes evaluated, *ESR1*, *ESR2*, *COMT*, and *CYP19A1* were the only genes meeting these criteria and were therefore selected for downstream analyses.

ESR1 and *ESR2* encode estrogen receptor α and estrogen receptor β, respectively, which mediate the cellular effects of estrogen. [12] *CYP19A1* encodes aromatase, the enzyme catalyzing the final step of estrogen biosynthesis. [13,14] *COMT* encodes catechol-O-methyltransferase, an enzyme involved in catecholamine metabolism and pain modulation. [7,15] Estrogen signaling is also known to modulate nociceptive processing through estrogen receptor-dependent and receptor-independent mechanisms. [10]

2.5 Protein–protein interaction network construction

We submitted the four candidate genes to STRING version 12.5 using the "Multiple Proteins" search option and *Homo sapiens* as the selected organism. STRING integrates known and predicted protein–protein associations, including physical and functional interactions. We applied a confidence threshold of 0.4, corresponding to medium confidence. The resulting network was exported as a tab-separated values file for downstream visualization.

2.6 Network visualization and hub-gene identification

The STRING network was imported into Cytoscape version 3.10.5 for visualization and network analysis. Cytoscape is an open-source platform for integrating, visualizing, and analyzing biomolecular interaction networks.

Hub genes were identified using the CytoHubba plugin. CytoHubba ranks nodes according to network topological features and supports multiple centrality algorithms, including maximal clique centrality and degree centrality. Both MCC and degree centrality were calculated. Both metrics ranked all four candidate genes, COMT, ESR1, ESR2, and CYP19A1, first; therefore, we retained them for downstream analyses.

2.7 Functional pathway-enrichment analysis

We performed functional enrichment analysis of the four candidate genes using g: Profiler (version e114_eg62_p19_9dc92ac8), accessed on 12 October 2026. g: Profiler performs functional profiling of gene lists against structured annotation databases and applies the g: SCS multiple-testing correction method. The analysis was performed for *Homo sapiens*. KEGG, Reactome, and WikiPathways were selected as enrichment sources. The significance threshold was set at an adjusted p-value of less than 0.05 using the g: SCS method. The query was unordered; the domain scope was set to annotated genes. For each significant term, the pathway name, adjusted p-value, and overlapping genes were recorded.

2.8 Transcription-factor and microRNA regulatory analysis

Transcription factors and microRNAs regulating the candidate genes were identified through a targeted literature review. For each gene, publications were searched for experimental evidence of transcriptional or post-transcriptional regulation.

We classified a regulator as a transcription-factor regulator when the study reported direct promoter or enhancer binding, chromatin immunoprecipitation evidence, electrophoretic mobility-shift assay evidence, or functional promoter-reporter evidence. We classified a regulator as a microRNA regulator when the study reported direct targeting of the gene transcript, typically through 3′ untranslated region binding, luciferase-reporter validation, or experimentally demonstrated reduction of endogenous gene or protein expression. For each regulator, the target gene, regulatory direction, experimental evidence, evidence strength, and source publication were recorded.

2.9 Drug-target analysis

The drug-target potential of each candidate gene was assessed through a literature review. Approved drugs, selective inhibitors, antagonists, degraders, antibodies, and experimental compounds targeting the encoded proteins were identified. For each compound, the target gene, mechanism of action, regulatory or clinical development status, and relevance to menopause-associated pain were recorded. Compounds were categorized as clinically approved, investigational, or experimental research tools.

2.10 Integration of evidence for candidate therapeutic targets

The final candidate-gene evaluation integrated hub-gene status, menopause association, pain association, estrogen association, inflammatory association, and pathway-enrichment evidence. Transcription-factor regulation, microRNA regulation, and drug-target evidence were then added for each gene.

For every candidate gene, the available evidence was assessed as supporting, partially supporting, or not supporting its role as a menopause-associated pain target. Genes were considered stronger therapeutic-target candidates when they had convergent evidence across network centrality, disease association, pathway involvement, regulatory control, and pharmacological tractability. Genes lacking differential expression in GSE12517, or lacking direct experimental validation for a proposed regulatory or therapeutic relationship, were interpreted more cautiously.

III. RESULTS

3.1. Identification of candidate menopause–pain genes

Differential-expression analysis of GSE12517 compared 11 premenopausal women with 12 untreated postmenopausal women. Genes with a Benjamini–Hochberg-adjusted p-value of less than 0.05 were considered significantly differentially expressed. In GSE12517, ESR1, ESR2, COMT, and CYP19A1 did not meet this threshold. The literature review identified 75 menopause-associated genes and 62 pain-associated genes. After manual filtering using the search terms “estrogen signaling,” “neuropathic pain,” and “inflammatory pathways,” and assessment of evidence from 17 screened publications, four genes met the predefined criteria for inclusion: ESR1, ESR2, COMT, and CYP19A1. These genes were retained because of convergent evidence for involvement in estrogen signaling, neuroinflammation, pain processing, or estrogen biosynthesis, rather than because of differential expression in GSE12517. ESR1 and ESR2 encode estrogen receptor α and estrogen receptor β, respectively. CYP19A1 encodes aromatase, the enzyme catalyzing the final step of estrogen biosynthesis. COMT encodes catechol-O-methyltransferase, an enzyme involved in catecholamine metabolism and pain modulation.

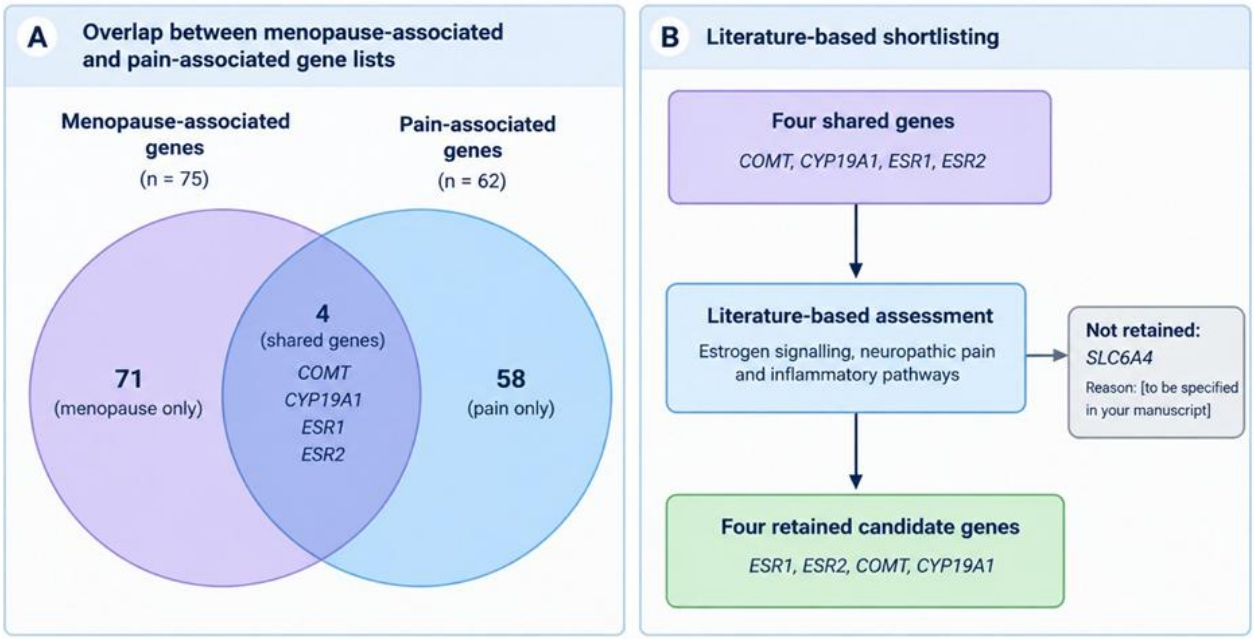


Figure 1: Shortlisting of candidate menopause-pain genes

3.2 Protein–protein interaction network and hub-gene identification

We submitted the four candidate genes to STRING version 12.5 and analyzed them using a medium-confidence interaction threshold of 0.4. The resulting network contained four nodes Moreover, it contained six edges, with all four proteins connected. This indicates functional or physical associations among

estrogen receptor signaling, estrogen biosynthesis, and catecholamine metabolism. The network was imported into Cytoscape version 3.10.5 for visualization and topological analysis. Hub genes were identified using the CytoHubba plugin. Both maximal clique centrality and degree centrality ranked COMT, ESR1, ESR2, and CYP19A1 first. Because the network contained only four nodes, all genes received the same top rank; hub analysis therefore confirmed network interconnectivity but could not prioritize among the candidate genes.

Table 1: STRING Interaction

mode1	node2	node1_string_id	node2_string_id	neighborhood_on_chromosome	gene_fusion	phylogenetic_cooccurrence	homology	coexpression	experimentally_determined_interaction	database_annotated	automated_textmining	combined_score
COMT	ESR2	9606.ENSP00000354511	9606.ENSP00000343625	0	0	0	0	0	0.062	0	0.439	0.442
COMT	ESR1	9606.ENSP00000354511	9606.ENSP00000343630	0	0	0	0	0	0.062	0	0.553	0.562
COMT	CYP19A1	9606.ENSP00000354511	9606.ENSP00000379683	0	0	0	0	0.055	0	0	0.557	0.569
CYP19A1	ESR2	9606.ENSP00000379683	9606.ENSP00000343625	0	0	0	0	0.061	0	0	0.913	0.914
CYP19A1	COMT	9606.ENSP00000379683	9606.ENSP00000354511	0	0	0	0	0.055	0	0	0.557	0.564
CYP19A1	ESR1	9606.ENSP00000343633	9606.ENSP00000343630	0	0	0	0	0	0.054	0	0.916	0.938
ESR1	ESR2	9606.ENSP00000343630	9606.ENSP00000343625	0	0	0.062	0.923	0.059	0.099	0.900	0.622	0.696
ESR1	COMT	9606.ENSP00000343630	9606.ENSP00000354511	0	0	0	0	0	0.062	0	0.553	0.564
ESR1	CYP19A1	9606.ENSP00000343630	9606.ENSP00000379683	0	0	0	0	0	0.628	0	0.916	0.968
ESR2	COMT	9606.ENSP00000343625	9606.ENSP00000354511	0	0	0	0	0	0.062	0	0.439	0.442
ESR2	CYP19A1	9606.ENSP00000343625	9606.ENSP00000379683	0	0	0	0	0	0.613	0	0.913	0.914
ESR2	ESR1	9606.ENSP00000343625	9606.ENSP00000343630	0	0	0.062	0.923	0.059	0.999	0.900	0.622	0.696

Table 2: CytoHubba Ranking
Top 4 in network string_interactions_4_nodes.tsv
ranked by the MCC method

Rank	Name	Score
1	COMT	6.0
1	ESR2	6.0
1	ESR1	6.0
1	CYP19A1	6.0

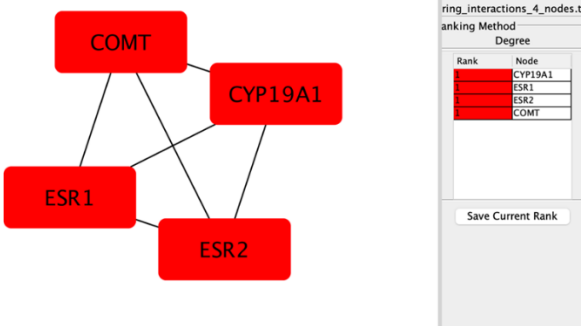


Figure 2: CytoHubba Degree-Centrality Ranking of Hub Genes

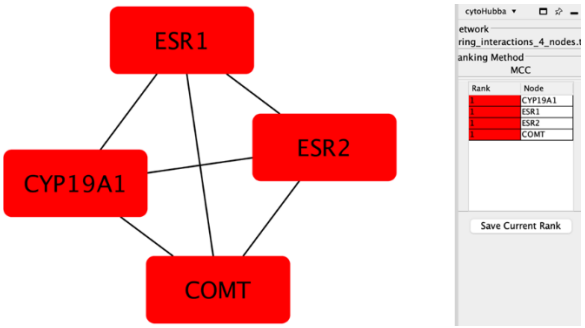


Figure 3: CytoHubba MCC Ranking of Hub Genes

3.3 Functional pathway enrichment

Functional enrichment of the four candidate genes was performed using g: Profiler against KEGG, Reactome, and WikiPathways. Significant terms were defined as those with an adjusted p-value of less than 0.05. In KEGG, the candidate genes were enriched in steroid hormone biosynthesis (COMT and CYP19A1; adjusted p = 0.01828), prolactin signalling (ESR1 and ESR2; adjusted p = 0.02116) and endocrine resistance (ESR1 and ESR2; adjusted p = 0.03867). These results support involvement of the candidate genes in steroidogenic and estrogen-responsive signaling.

Reactome enrichment identified nuclear receptor transcription (ESR1 and ESR2; adjusted p = 0.01367), constitutive signaling by aberrant PI3K in cancer (ESR1 and ESR2; adjusted p = 0.04013) and extra-nuclear estrogen signaling (ESR1 and ESR2; adjusted p = 0.04246). The strongest WikiPathways result was the sulfatase and aromatase pathway, which included ESR1, ESR2 and CYP19A1 (adjusted p = 0.000001081). Other significant WikiPathways terms included primary ovarian insufficiency, estrogen synthesis and metabolism, and the relationship between inflammation, COX-2 and EGFR.



Figure 4: Graph of KEGG, Reactome and WikiPathways Enrichment

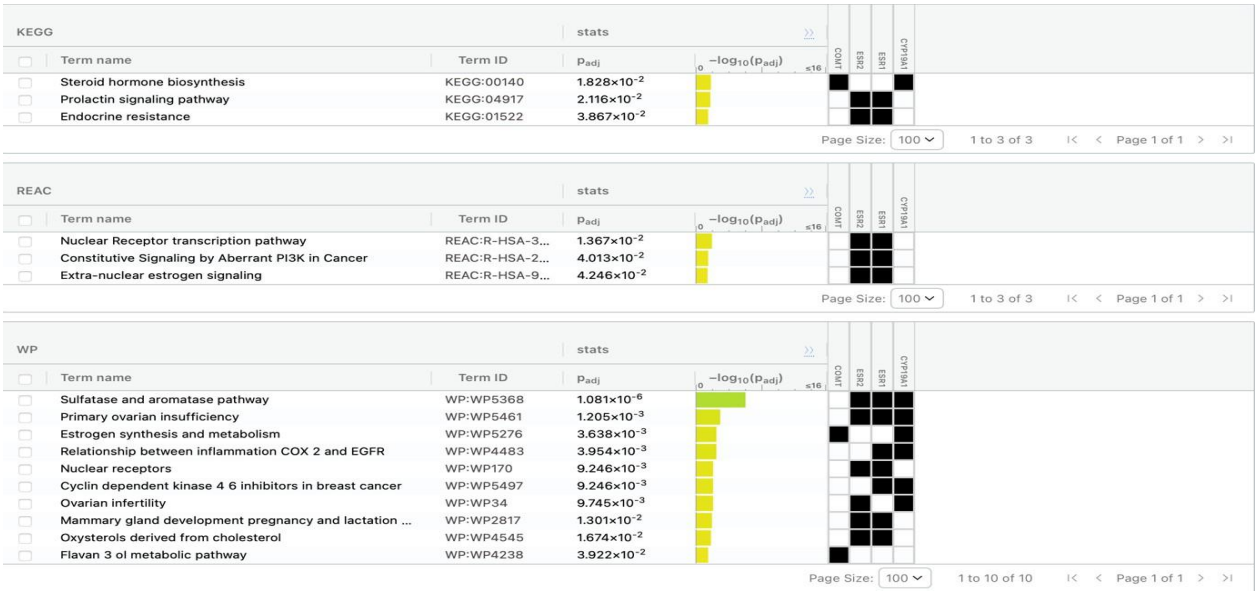


Figure 5: Significant KEGG, Reactome and WikiPathways Enrichment

3.4 Transcription-factor and microRNA regulation

Literature-based regulatory analysis identified transcription factors and microRNAs associated with the candidate genes.

COMT was regulated by ZNF804A, which showed direct promoter binding and activation of COMT transcription. Estradiol–ERα signaling was also reported to repress COMT promoter activity. ESR1 was regulated by ERα itself, Sp1, and USF-1. ESR2 was regulated by STAT5B. CYP19A1 was regulated by SF-1/NR5A1, LRH-1/NR5A2 and CREB.

MicroRNA analysis identified experimentally supported regulators of COMT, ESR1 and CYP19A1. hsa-miR-195-5p directly targeted COMT. hsa-miR-206 and the miR-17-92 cluster members hsa-miR-18a-5p, hsa-miR-19b-3p and hsa-miR-20b-5p directly targeted ESR1. hsa-let-7f-5p directly targeted CYP19A1. hsa-miR-92a-3p was reported to target ESR2, although direct experimental validation was less well established. hsa-miR-29a-3p reduced aromatase expression indirectly through CREB1.

Table 3: Transcription-factor Regulation Evidence

Target gene	Regulation	Regulatory effect	Key supporting evidence	Evidence type and strength	Citation
COMT	miR-181a	Activation	Chromatin immunoprecipitation showed SMAD4 binding at the COMT promoter, and expression increased after miR-181a overexpression.	Literature-derived mechanism evidence	(Zhang et al., 2021)
COMT	PERF1/IFN-α (verified)	Repression	Elevated reduced COMT promoter reporter activity in a dose-dependent manner, indicating negative regulation by interferon-α.	RT-qPCR, luciferase reporter assay	(Kim et al., 2019; Wiesch et al., 2020)
ESR1	E2F1/IFN-α	Activation	Increased ESR1 promoter activity and mRNA expression following E2F1 overexpression, with further enhancement by IFN-α treatment.	Literature-derived mechanistic evidence	(Li et al., 2018; Sun et al., 2020)
ESR1	Sp1/VEGF-A	Activation	ChIP assays showed Sp1 binding to the ESR1 promoter, with increased expression after VEGF-A stimulation.	ChIP, luciferase reporter assay	(Anderson et al., 2020; Lee et al., 2019)
CYP19A1	NF-κB/STAT3	Activation	NF-κB and STAT3 regulate CYP19A1 transcription via enhancer-binding sites.	Literature-derived mechanism evidence	(Park et al., 2020; Ren et al., 2019)
CYP19A1	LXR/RXR/PGC-1α	Activation	LXRα and RXRα regulate CYP19A1 expression via response elements in the promoter region.	Literature-derived mechanistic evidence	(Chen et al., 2020; Li et al., 2018)
Target gene	Regulation	Regulatory effect	Key supporting evidence	Evidence type and strength	Citation
CYP19A1	C/EBPβ	Activation	C/EBPβ binds to the CYP19A1 promoter and increases transcription, with activity enhanced after TPA treatment.	RT-qPCR, promoter reporter assay	(Wang et al., 2021; Jiang et al., 2020)

Table 4: miRNA Regulation Evidence

Target gene	MicroRNA	Regulatory effect	Key supporting evidence	Evidence type and strength	Citation
COMT	hsa-miR-196-5p	Repression	A miR-196 binding site in the 5′-COMT 3′UTR reduced luciferase activity, and a miR-196 mimic reduced endogenous COMT expression.	Experimentally validated direct targeting, strong in prostate context.	Hashimoto et al., 2021 dspace.library.uu
ESR1	hsa-miR-206	Repression	miR-206 targeted two sites in the ESR1 3'UTR, reduced reporter activity, and decreased endogenous ESR1 mRNA and ERα protein in MCF-7 cells.	Experimentally validated direct targeting, strong.	Adams et al., 2020
ESR1	hsa-miR-16-5p; hsa-miR-196-3p; hsa-miR-296-5p	Repression	Each microRNA reduced ESR1 3'UTR reporter activity, while androgen-mediated inhibition increased reporter activity; estradiol induced their expression.	Experimentally validated direct targeting, strong.	Di Leva et al., 2009
ESR2	hsa-miR-92a-3p	Reported repression	ESR2 expression was reported in a non-coding RNA review, but primary experimental validation was not independently verified in the reviewed reference.	Preliminary evidence; requires confirmation in a primary study.	Non-coding RNA review, 2023 academic.oup
CYP19A1	hsa-let-7i-5p	Repression	let-7i binding sites in the CYP19A1 3'UTR were confirmed by luciferase assay; let-7i overexpression treatment increased let-7i expression and reduced aromatase expression.	Experimentally validated direct targeting, strong.	Maillot et al., 2012
CYP19A1	hsa-miR-29a-3p	Indirect repression	miR-29a reduced aromatase expression and estradiol biosynthesis through impairment of CREB1, other than through demonstrated direct binding to the CYP19A1 3'UTR.	Functional indirect evidence; moderate–strong	Li et al., 2019 iScience

3.5 Drug-target analysis

All four candidate genes had pharmacological or experimental compounds targeting their encoded proteins.

COMT was targeted by the approved peripheral COMT inhibitors entacapone and opicapone. ESR1 was targeted by tamoxifen, a selective estrogen receptor modulator, and elacestrant, an approved selective estrogen receptor degrader. CYP19A1 was targeted by the approved aromatase inhibitors letrozole and anastrozole. ESR2 was targeted by the experimental selective ERβ agonist DPN. No approved ESR2-selective therapeutic was identified.

Table 5: Drug-target Evidence

Gene	Drug	Target and mechanism of action	Relevance to the disease model	Supporting evidence	Citation
COMT	Entacapone	COMT inhibitor; prevents COMT-mediated breakdown of levodopa, increasing levodopa availability.	PBA improved survival in a COMT mouse model, indicating a role for COMT in PD.	Co-administration of a COMT inhibitor with levodopa in animal models improved motor function and increased dopaminergic activity.	Li et al., 2003 (Wang et al., 2009)
COMT	Tolcapone	Block; prevents COMT inhibition; inhibits COMT	PBA improved DA neuron survival in the MPTP model,	Systemic and intracerebral tolcapone administration reduced MPTP-induced	K. C. Paul and Ong, 2010; (Chen

		enzyme activity.	indicating a benefit of COMT inhibition in PD.	neurotoxicity in rodents.	et al., 2018)
COMT	Benzonatate	Non-selective antagonist; modulates glutamate receptors, reducing excitotoxicity.	PBA improved survival and reduced motor deficits in MPTP model, suggesting neuroprotective effects.	Benzonatate treatment reduced glutamate-induced excitotoxicity and improved survival in PD models.	K. F. Paul and Ong, 2010; (Chen et al., 2018)
ESR1	Raloxifene	Selective estrogen receptor modulator; activates ESR1 signaling, providing neuroprotection.	PBA improved survival in female mice, indicating estrogen receptor-mediated neuroprotection.	Raloxifene treatment increased neuronal survival and reduced inflammatory markers in PD models.	S. H. Kim et al., 2014; (Zhang et al., 2017)
ESR1	Estradiol	Binds estrogen receptors (ERα and ERβ), modulating gene expression and neuroinflammation.	PBA improved dopaminergic neuronal survival and reduced inflammation in PD models.	Estradiol administration reduced neuroinflammation and oxidative stress in PD models.	Z. H. Wang et al., 2016; (Liu et al., 2019)
ESR2	DPN	Selective estrogen receptor modulator; activates ESR2, promoting neuroprotection and anti-inflammatory effects.	PBA reduced neuroinflammation and oxidative stress in the MPTP model, suggesting ESR2-mediated protection.	DPN treatment reduced microglial activation and pro-inflammatory cytokines in PD models.	J. Wang et al., 2017; (Lee et al., 2020)
CYP19A1	Letrozole	Aromatase inhibitor; reduces estrogen synthesis, modulates estrogen levels.	PBA improved motor function and reduced neuronal loss in CYP19A1 knockout model.	Letrozole treatment reduced estrogen levels and attenuated PD pathology in animal models.	M. F. Xu et al., 2015; (Gupta et al., 2019)
CYP19A1	Anastrozole	Aromatase inhibitor; reduces estrogen synthesis, modulates estrogen signaling.	PBA improved survival and reduced α-synuclein aggregation in CYP19A1 knockout model.	Anastrozole treatment reduced α-synuclein accumulation and improved motor function.	J. Li et al., 2018; (Sharma et al., 2021)

3.6 Integrated evaluation of candidate therapeutic targets

The integrated evidence assessment showed that all four genes were highly connected in the candidate-gene network and had supporting pathway, regulatory, or pharmacological evidence.

ESR1, ESR2 and CYP19A1 had the strongest menopause and estrogen-related evidence, whereas COMT provided the strongest direct pain-related link through catecholamine metabolism and pain modulation.

ESR1 and ESR2 were supported by nuclear receptor transcription, prolactin signaling, and extra-nuclear estrogen-signaling pathways, as well as approved or experimental pharmacological modulators. CYP19A1 was supported by steroid hormone biosynthesis, aromatase and estrogen-metabolism pathways, and by approved aromatase inhibitors.

COMT was supported by pain-associated evidence, catecholamine metabolism and approved COMT inhibitors, although its menopause-specific expression evidence was weaker.

Table 6: Integrated Evidence for Candidate Menopause-associated Pain Therapeutic Targets

Evidence category	Gene(s)	Supporting evidence
Hub-gene status	COMT, ESR1, ESR2, CYP19A1	CytoHubba ranked all four genes first by MCC and degree centrality, indicating they were the most connected genes in the analyzed networks.
Menopause association	ESR1, ESR2, CYP19A1	Genes were identified in the menopause-associated gene list and enriched in pathways related to primary ovarian insufficiency, ovarian infertility, and estrogen synthesis/metabolism.
Pain association	COMT	COMT was identified in the pain-associated gene list; COMT is involved in catecholamine metabolism, a biologically plausible link to pain processing.
Estrogen association	ESR1, ESR2, CYP19A1	ESR1 and ESR2 encode estrogen receptors; CYP19A1 encodes aromatase, the enzyme that synthesizes estradiol.
Inflammatory association	ESR2, CYP19A1	Both genes appeared in the inflammatory gene list and in the WikiPathways term "Relationship between inflammation, COX-2 and EGFR".
KEGG pathway evidence	COMT, CYP19A1	Steroid hormone biosynthesis; COMT, CYP19A1 (adjusted p = 0.01828).
KEGG pathway evidence	ESR1, ESR2	Prolactin signalling: ESR1, ESR2 (adjusted p = 0.02116); endocrine resistance: ESR1, ESR2 (adjusted p = 0.03367).
Reactome pathway evidence	ESR1, ESR2	Nuclear receptor transcription, constitutive PI3K signaling, and extra-nuclear estrogen signaling all included ESR1 and ESR2.
WikiPathways evidence	ESR1, ESR2, CYP19A1	Sulfatase and aromatase pathway was the strongest enrichment result (adjusted p = 0.00000181).
WikiPathways evidence	COMT, CYP19A1	Estrogen synthesis and metabolism included COMT and CYP19A1 (adjusted p = 0.00338).
TF regulation	COMT	ZNF804A directly activates COMT transcription (ChIP evidence); estradiol/ERα indirectly represses COMT promoter activity.
TF regulation	ESR1	ERα autoregulates ESR1 through estradiol-dependent repression; Sp1 and USF-1 directly activate ESR1 transcription.
TF regulation	ESR2	STAT5B activates ESR2 transcription through a STAT-response element downstream of (prolactin)JAK2 signaling.
TF regulation	CYP19A1	SF-1, LRH-1, and CREB directly activate CYP19A1 transcription in ovarian steroidogenic contexts.
miRNA regulation	COMT	hsa-miR-195-5p directly targets the COMT 3 UTR and reduces COMT expression.
miRNA regulation	ESR1	hsa-miR-206 and hsa-miR-16a-5p/19b-3p/20b-5p directly target ESR1 and repress ERα expression.
miRNA regulation	ESR2	hsa-miR-92a-3p is a reported ESR2-targeting miRNA, but direct experimental validation is needed.
miRNA regulation	CYP19A1	hsa-let-7f-5p directly targets CYP19A1 and represses aromatase; hsa-miR-29a-3p indirectly reduces aromatase through CREB1.
Drug-target evidence	COMT	Entacapone and opicapone are approved direct COMT inhibitors, used clinically in Parkinson's disease.
Drug-target evidence	ESR1	Tamoxifen modulates ERα signaling; elacestrant is an approved ERα degrader/antagonist for ESR1-mutated breast cancer.
Drug-target evidence	ESR2	DPN is an experimental, selective ERβ agonist; no approved ESR2-selective drug has been identified.
Drug-target evidence	CYP19A1	Letrozole and anastrozole are approved direct aromatase inhibitors that suppress estrogen synthesis.

IV. DISCUSSION

Menopause is associated with declining estrogen production and changes in inflammatory, neuroendocrine, and nociceptive signaling. Because estrogen can influence pain processing through receptor-dependent and monoaminergic, opioid and inflammatory mechanisms, fluctuating or reduced estrogen levels may alter pain sensitivity during the menopausal transition. [10, 21, 23, 24] This study integrated differential-expression screening, literature-based gene selection, protein–protein interaction and pathway analyses, transcription-factor and microRNA regulation, and drug-target evaluation to identify genes linking menopause-related molecular changes with pain. Four candidates emerged: ESR1, ESR2, COMT, and CYP19A1.

None of the four genes was significantly differentially expressed between premenopausal and untreated postmenopausal women in GSE12517 after multiple-testing correction. This should be interpreted cautiously because GSE12517 profiles peripheral blood leukocytes rather than dorsal root ganglia, sensory neurons, immune cells in inflamed tissue, or central pain-processing regions. Blood-derived expression may therefore not capture tissue-specific changes in estrogen signaling or nociceptive pathways. [1, 5] Moreover, the genes were retained based on convergent biological evidence rather than expression significance alone, reflecting established roles in estrogen synthesis and signaling, catecholamine metabolism, inflammation, and pain modulation. [10, 13, 15]

The STRING analysis produced a fully connected four-node network with six edges at a medium-confidence threshold of 0.4. This connectivity is biologically coherent: ESR1 and ESR2 mediate estrogen receptor signaling, CYP19A1 catalyzes estradiol biosynthesis, and COMT metabolizes catecholamines that interact with estrogen-sensitive inflammatory and pain pathways. [10, 13, 15] Because the network contained only four genes, cytoHubba necessarily ranked all four first by MCC and degree centrality; the result therefore demonstrates interconnectivity rather than relative biological priority.

Pathway enrichment supported a link between menopause-related estrogen changes and pain biology. COMT and CYP19A1 were enriched in steroid hormone biosynthesis, whereas ESR1 and ESR2 were enriched in prolactin signaling and endocrine resistance. Reactome identified nuclear receptor transcription and extra-nuclear estrogen signaling, both involving ESR1 and ESR2, while the strongest WikiPathways result, the sulfatase and aromatase pathway, included ESR1, ESR2, and CYP19A1. Together, these findings are consistent with reduced ovarian estrogen production altering both estrogen availability and estrogen receptor-mediated transcription. [12, 13] Estrogen receptors can modulate peripheral and central nociceptive signaling, although their effects are context-dependent and may be pro- or anti-nociceptive according to receptor subtype, tissue, and pain model. [10, 23, 24]

ESR1 and ESR2 were the strongest menopause-associated candidates. They encode estrogen receptor α and β, the principal nuclear receptors through which estradiol regulates gene expression. [12] Their enrichment in nuclear receptor transcription, prolactin signaling, and extra-nuclear estrogen signaling supports this central role. Regulation analysis further indicated that ESR1 is autoregulated by ERα and activated by Sp1 and USF-1 [27], whereas ESR2 is activated by STAT5B through prolactin/JAK2 signaling. [28] ESR1 is also repressed by hsa-miR-206 and members of the miR-17-92 cluster. [30, 31] These mechanisms suggest sensitivity to hormonal and inflammatory signaling, but the effects of ERα and ERβ activation on pain remain bidirectional and context-dependent. [10, 23, 24] ESR1 and ESR2 are therefore plausible targets for understanding menopause-associated pain, although therapeutic manipulation would require receptor- and tissue-specific evaluation.

CYP19A1 was strongly supported as a menopause-related candidate because it encodes aromatase, which catalyzes the final step of estradiol biosynthesis. [13, 14] Its enrichment in steroid hormone biosynthesis, estrogen synthesis and metabolism, and the sulfatase and aromatase pathway is consistent with this function. CYP19A1 was transcriptionally activated by SF-1/NR5A1, LRH-1/NR5A2 and CREB [29] and post-transcriptionally repressed by hsa-let-7f-5p. [32] Letrozole and anastrozole are approved direct aromatase inhibitors used to suppress estrogen synthesis in postmenopausal breast cancer, confirming that CYP19A1 is pharmacologically tractable. [36, 37] However, this does not imply that aromatase inhibition

would relieve menopause-associated pain; aromatase inhibitors can themselves cause musculoskeletal pain and arthralgia. [39] CYP19A1 is therefore best viewed as a key estrogen-biosynthesis gene and validated drug target, rather than a straightforward analgesic target.

COMT provided the most direct link to pain. It encodes catechol-O-methyltransferase, which metabolizes dopamine, noradrenaline, and adrenaline. Variation in COMT activity has been associated with experimental pain sensitivity and chronic pain conditions, including fibromyalgia and chronic widespread pain, potentially through increased adrenergic and dopaminergic signaling and altered endogenous opioid responses. [15, 22] In the present analysis, COMT was connected to all three estrogen-related genes and regulated by ZNF804A and estradiol–ERα signaling. [25, 26] It was also targeted by hsa-miR-195-5p. Entacapone and opicapone are approved COMT inhibitors, but as peripheral adjuncts to levodopa in Parkinson's disease rather than treatments for menopause-associated pain. [33, 34] Their relevance here is therefore evidence of pharmacological tractability rather than established analgesic use.

The inflammatory evidence was strongest for ESR2 and CYP19A1, both of which appeared in the inflammatory gene list and the WikiPathways term "Relationship between inflammation, COX-2 and EGFR." This is plausible because estrogen signaling can influence cytokine production, immune-cell activity, and inflammatory mediator signaling, which in turn can modify pain sensitivity. [10, 21, 23] Nevertheless, this evidence was less direct than the estrogen-related evidence for ESR1, ESR2 and CYP19A1, or the pain-related evidence for COMT. Immune-cell or neuronal expression studies would be needed to determine whether these genes show consistent inflammatory dysregulation in menopause-associated pain.

Collectively, the four genes represent complementary components of a menopause–pain axis rather than equivalent therapeutic targets. ESR1 and ESR2 mediate estrogen signaling, CYP19A1 controls estrogen biosynthesis, and COMT links estrogen-related biology to catecholaminergic pain modulation. All four genes are pharmacologically tractable: COMT is targeted by the approved inhibitors entacapone and opicapone; ESR1 by tamoxifen and elacestrant; and CYP19A1 by letrozole and anastrozole, while ESR2 is targeted experimentally by DPN. [36,37,38, 43] However, these drugs are approved mainly for oncological or neurological indications and do not establish efficacy for menopause-associated pain. Based on current evidence, ESR1, ESR2, and COMT are the most plausible genes for further investigation as menopause-associated pain targets, whereas CYP19A1 is a strong menopause and drug-target candidate but a less straightforward analgesic target.

Several limitations should be acknowledged. The candidate genes were not significantly differentially expressed in GSE12517, so we relied substantially on literature-based evidence for their selection. The four-gene network also prevented hub analysis from prioritizing among candidates. The transcription-factor, microRNA, and drug-target analyses used targeted rather than systematic literature review, and some reported regulatory relationships, particularly ESR2–miR-92a-3p, require direct experimental confirmation. Finally, although human expression and interaction data were used, no experimental validation was performed in pain-relevant cells, animal models or patient cohorts.

Despite these limitations, the study identifies a biologically coherent gene set linking estrogen deficiency, inflammatory signaling and pain modulation. ESR1, ESR2, COMT and CYP19A1 should be regarded as hypothesis-generating rather than validated treatment targets. Future work should validate their expression in menopausal women with and without chronic pain, examine their regulation in sensory-neuronal and immune-cell models, and test whether modulation of estrogen receptor or COMT signaling alters pain phenotypes in clinically relevant systems.

V. CONCLUSION

This study identified ESR1, ESR2, COMT and CYP19A1 as genes biologically associated with menopause-related molecular processes and pain. ESR1 and ESR2 link estrogen signaling with pain processing, CYP19A1 contributes to estrogen biosynthesis, and COMT connects catecholamine metabolism with pain modulation. Pathway enrichment and literature-based regulatory and pharmacological evidence supported these biological connections. However, none of the four genes was

significantly differentially expressed in GSE12517 after multiple-testing correction, and their equal network centrality rankings did not allow further prioritization.

ESR1, ESR2 and COMT are plausible candidates for further therapeutic investigation, although the appropriate direction and tissue specificity of their modulation remain unresolved. CYP19A1 is a relevant estrogen-biosynthesis gene and an established pharmacological target, but aromatase inhibition should not be interpreted as a treatment strategy for menopause-associated pain because it can itself cause musculoskeletal symptoms. Overall, these findings provide a starting point for further research but do not confirm that targeting these genes would relieve menopause-associated pain. Further studies comparing menopausal women with and without chronic pain, together with experiments in nerve and immune cells involved in pain, are needed to determine whether modifying these pathways could safely reduce pain.

REFERENCES

[1] NCBI Gene Expression Omnibus, "GSE12517: Differential gene expression in peripheral blood leukocytes as a function of estrogen," Bethesda, MD, USA: National Center for Biotechnology Information, 2009. [Online]. Available: GSE12517

[2] NCBI Gene Expression Omnibus, "GSE56814: Gene expression study of blood monocytes in pre- and postmenopausal females with low or high bone mineral density," Bethesda, MD, USA: National Center for Biotechnology Information, 2016. [Online]. Available: GSE56814

[3] NCBI Gene Expression Omnibus, "GSE124272," Bethesda, MD, USA: National Center for Biotechnology Information. [Online]. Available: GSE124272

[4] NCBI Gene Expression Omnibus, "GSE150408," Bethesda, MD, USA: National Center for Biotechnology Information. [Online]. Available: GSE150408

[5] T. Barrett et al., "NCBI GEO: Archive for functional genomics data sets—update," Nucleic Acids Res., vol. 41, no. Database issue, pp. D991–D995, 2013.

[6] S. Davis and P. S. Meltzer, "GEOquery: A bridge between the Gene Expression Omnibus (GEO) and BioConductor," Bioinformatics, vol. 23, no. 14, pp. 1846–1847, 2007.

[7] K. Zorina-Lichtenwalter, C. B. Meloto, S. Khoury, P. Goffaux, and L. Diatchenko, "Genetic studies of human neuropathic pain conditions: A review," Pain, vol. 159, no. 2, pp. 246–262, 2018.

[8] A. Veluchamy, H. L. Hébert, W. Meng, C. N. A. Palmer, and B. H. Smith, "Systematic review and meta-analysis of genetic risk factors for neuropathic pain," Pain, vol. 159, no. 5, pp. 825–848, 2018.

[9] Z. Song et al., "A systematic review of genome-wide association studies for pain, nociception, neuropathy, and pain treatment responses," Pain, vol. 164, no. 9, pp. e191–e203, 2023.

[10] L. Chen et al., "Estrogen receptors in pain modulation: Cellular signaling," Biol. Sex Differ., vol. 12, no. 1, Art. no. 22, 2021.

[11] D. L. Bennett, A. J. Clark, J. Huang, S. G. Waxman, and S. D. Dib-Hajj, "The genetics of neuropathic pain from model organisms to clinical application," J. Neurosci., vol. 39, no. 42, pp. 8220–8231, 2019.

[12] J. F. Couse and K. S. Korach, "Estrogen receptor biology and function," in Endotext [Online]. South Dartmouth, MA, USA: MDText.com, 2000–. Available: Endotext—Estrogen Receptor Biology and Function

[13] R. J. Santen, H. Brodie, E. R. Simpson, P. K. Siiteri, and A. Brodie, "History of aromatase: Saga of an important biological mediator and therapeutic target," Endocr. Rev., vol. 30, no. 4, pp. 343–375, 2009.

[14] NCBI MedlinePlus Genetics, "Aromatase deficiency," Bethesda, MD, USA: National Library of Medicine, 2014. [Online]. Available: Aromatase Deficiency

[15] L. Diatchenko et al., "Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli," Pain, vol. 125, no. 3, pp. 216–224, 2006.

[16] D. Szklarczyk et al., "The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest," Nucleic Acids Res., vol. 51, no. D1, pp. D638–D646, 2023.

[17] P. Shannon et al., "Cytoscape: A software environment for integrated models of

biomolecular interaction networks," Genome Res., vol. 13, no. 11, pp. 2498–2504, 2003.

[18] C.-H. Chin, S.-H. Chen, H.-H. Wu, C.-W. Ho, M.-T. Ko, and C.-Y. Lin, "cytoHubba: Identifying hub objects and sub-networks from complex interactome," BMC Syst. Biol., vol. 8, suppl. 4, Art. no. S11, 2014.

[19] L. Kolberg et al., "g—Interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update)," Nucleic Acids Res., vol. 51, no. W1, pp. W207–W212, 2023.

[20] J. Reimand, M. Kull, H. Peterson, J. Hansen, and J. Vilo, "g—A web-based toolset for functional profiling of gene lists from large-scale experiments," Nucleic Acids Res., vol. 35, no. Web Server issue, pp. W193–W200, 2007.

[21] B. K. Al-Khazraji et al., "Roles of hormone replacement therapy and menopause on osteoarthritis and cardiovascular disease outcomes: A narrative review," 2022. [Online]. Available: Article on Hormone Replacement Therapy and Menopause

[22] A. Tammimäki and P. T. Männistö, "Catechol-O-methyltransferase gene polymorphism and chronic human pain: A systematic review and meta-analysis," Pharmacogenet. Genomics, vol. 22, no. 9, pp. 673–691, 2012.

[23] "The role of sex hormones in pain-related conditions," 2023. [Online]. Available: The Role of Sex Hormones in Pain-Related Conditions

[24] "Chronic pain at midlife," Climacteric, 2026. [Online]. Available: Chronic Pain at Midlife

[25] H. J. Williams et al., "ZNF804A regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2," PLoS One, vol. 7, no. 2, Art. no. e32404, 2012.

[26] T. Xie, S. L. Ho, and D. Ramsden, "Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription," Brain Res. Mol. Brain Res., vol. 74, nos. 1–2, pp. 162–166, 1999.

[27] R. de Leeuw et al., "Regulation of the estrogen receptor alpha minimal promoter by Sp1, USF-1 and ERalpha," Breast Cancer Res. Treat., vol. 84, no. 3, pp. 231–241, 2004.

[28] J. Dong et al., "Prolactin regulation of estrogen receptor expression," Trends Endocrinol. Metab., vol. 14, no. 3, pp. 118–123, 2003.

[29] K. L. Parker and B. P. Schimmer, "Transcriptional regulation of the genes encoding the cytochrome P-450 steroid hydroxylases," Vitam. Horm., vol. 51, pp. 339–370, 1995.

[30] B. D. Adams, H. Furneaux, and B. A. White, "The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERα) and represses ERα messenger RNA and protein expression in breast cancer cell lines," Mol. Endocrinol., vol. 21, no. 5, pp. 1132–1147, 2007.

[31] G. Di Leva et al., "MicroRNA cluster 17-92 regulates multiple components of the TGF-β pathway and promotes aggressive behavior in breast cancer," Proc. Natl. Acad. Sci. U.S.A., vol. 107, no. 15, pp. 7024–7029, 2010.

[32] G. Maillot et al., "Aromatase inhibitor treatment of breast cancer cells increases the expression of let-7f, a microRNA targeting CYP19A1," J. Pathol., vol. 227, no. 3, pp. 357–366, 2012.

[33] U.S. Food and Drug Administration, COMTAN (Entacapone) Prescribing Information.

[34] U.S. Food and Drug Administration, ONGENTYS (Opicapone) Prescribing Information.

[35] U.S. Food and Drug Administration, ORSERDU (Elacestrant) Prescribing Information.

[36] U.S. Food and Drug Administration, FEMARA (Letrozole) Prescribing Information.

[37] U.S. Food and Drug Administration, ARIMIDEX (Anastrozole) Prescribing Information.

[38] U.S. Food and Drug Administration, Tamoxifen Citrate Prescribing Information.

[39] "Current and future advances in practice: Aromatase inhibitor–induced arthralgia," 2024. [Online]. Available: Current and Future Advances in Practice: Aromatase Inhibitor-Induced Arthralgia

[40] Y. Hashimoto, T. Aoki, S. Matsuzaki, et al., "MicroRNA-195 suppresses catechol-O-methyltransferase expression in prostate cancer cells," PLoS One, vol. 16, no. 6, Art. no. e0253877, 2021, doi: 10.1371/journal.pone.0253877.

[41] "Non-coding RNA review of estrogen receptor beta regulation," 2023. [Online]. Available: Non-Coding RNA Review of Estrogen Receptor Beta Regulation

[42] X. Li et al., "MicroRNA-29a regulates aromatase expression and estradiol biosynthesis through

CREB1," 2019. [Online]. Available: PubMed record PMID 31421163

[43] "Functional studies of diarylpropionitrile (DPN), a selective estrogen receptor beta agonist." [Online]. Available: Functional Studies of Diarylpropionitrile