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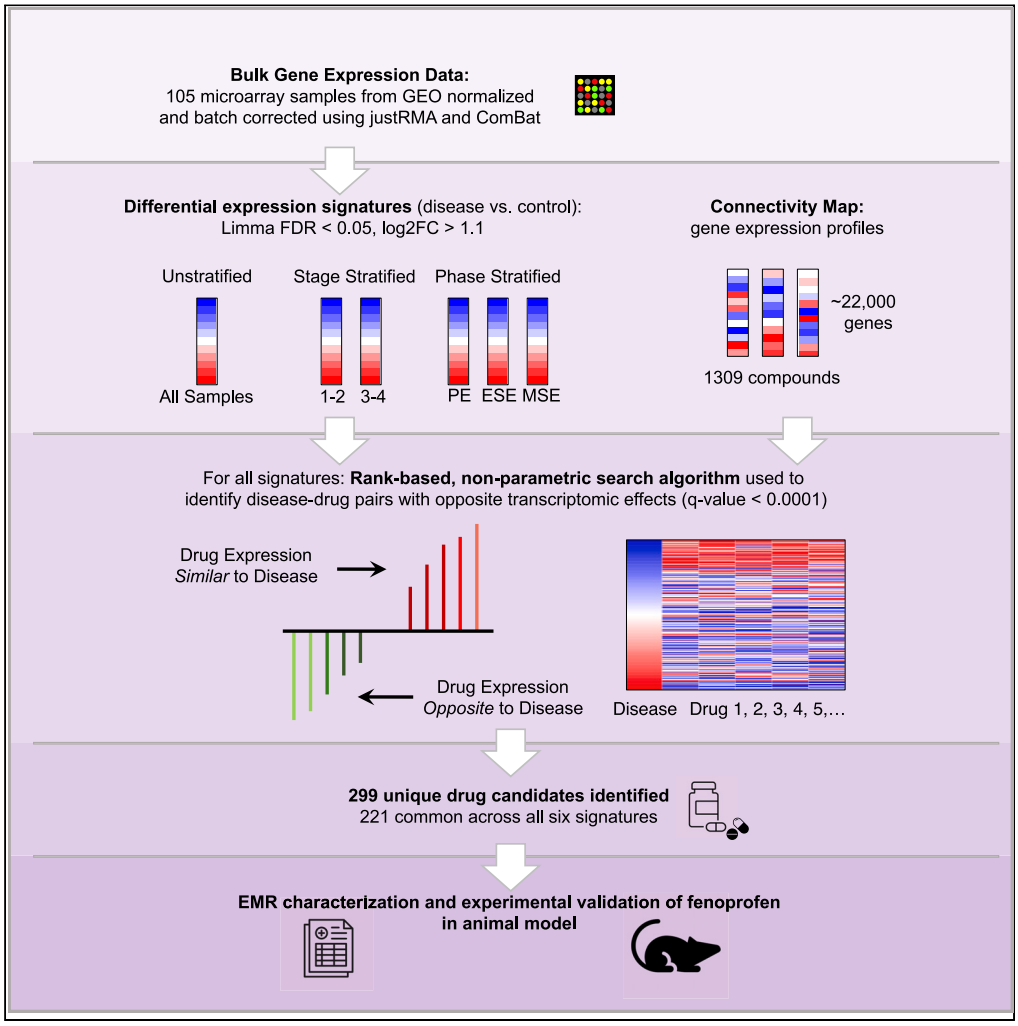

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Highlights

We used a computational drug repurposing method to identify endometriosis treatments

Rarely prescribed NSAID fenoprofen emerged as a potential endometriosis therapeutic

Fenoprofen alleviated vaginal hyperalgesia in a rat model of endometriosis

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Identifying therapeutic candidates for endometriosis through a transcriptomics-based drug repositioning approach

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SUMMARY

Existing medical treatments for endometriosis-related pain are often ineffective, underscoring the need for new therapeutic strategies. In this study, we applied a computational drug repurposing pipeline to stratified and unstratified disease signatures based on endometrial gene expression data to identify potential therapeutics from existing drugs, based on expression reversal. Of 3,131 unique genes differentially expressed by at least one of six endometriosis signatures, only 308 (9.8%) were in common; however, 221 out of 299 drugs identified, (73.9%) were shared. We selected fenoprofen, an uncommonly prescribed NSAID that was the top therapeutic candidate for further investigation. When testing fenoprofen in an established rat model of endometriosis, fenoprofen successfully alleviated endometriosis-associated vaginal hyperalgesia, a surrogate marker for endometriosis-related pain. These findings validate fenoprofen as a therapeutic that could be utilized more frequently for endometriosis and suggest the utility of the aforementioned computational drug repurposing approach for endometriosis.

INTRODUCTION

Endometriosis is an estrogen-dependent inflammatory condition characterized by the presence of endometrial-like tissue, refluxed during menses into the pelvis or, less commonly, by hematogenous or lymphatic spread to other parts of the body. It affects over 200 million people of reproductive age worldwide and up to 50% of those with infertility.[1] The most common symptom of endometriosis is pain, which can occur with any disease severity and at any menstrual cycle phase, and ~50% of women with chronic pelvic pain have endometriosis.[1,2] First-line treatment for endometriosis-associated pain involves nonsteroidal anti-inflammatory drugs (NSAIDs) and hormonal suppressive therapy with progestins, combined oral contraceptives, or GnRH agonists or antagonists.[3] However, these treatments are often ineffective, with nearly 19% of patients experiencing no reduction in pain and up to 59% having remaining pain,[4] thus making it essential to identify effective therapeutic candidates for endometriosis-related pain.

New drug development has been limited for endometriosis-related pain, likely due to numerous factors including the heterogeneity of the disease subtypes and presenting symptoms, and less investment globally in women's health related disorders, including those associated specifically with menstruation and pain.[5] Also, traditional drug development is time-consuming and expensive; it can take over 15 years and $1 billion to bring a new drug to market.[6] This is especially true of endometriosis due to the complexity of the disease, its etiology, and pathophysiology. It becomes essential, then, that alternate paths are pursued. Computational drug repurposing is the process of identifying therapeutic applications for existing compounds via computational methods. In recent years, large public datasets have been made possible by high throughput profiling technology, and efficient computation and analysis of big data have become more accessible. As a

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result, computational drug repurposing has gained traction as a modern innovation on traditional methodologies. Narrowing down candidates for experimental validation to existing drugs with transcriptomic profiles that suggest therapeutic effectiveness when applied to a disease mitigates the risk of failure in early stages of drug development. In addition, since every candidate is FDA-approved, identified drugs have already been subjected to clinical trials and have established safety and side effect profiles. This combination of factors vastly decreases time and cost, and shortens the path from initial development to clinical use.

One method of computational drug repurposing, pioneered by our group, uses a pattern-matching strategy to identify drugs and diseases with reversed differential gene expression profiles—where genes downregulated in a disease are upregulated by the drug treatment and vice versa. This approach relies on transcriptomics data, which can be leveraged to generate profiles of gene changes for both drugs and diseases. These profiles measure genome-wide changes in gene expression between an experimental state and a control state (in this case, a disease sample vs. a healthy control, or a drug-exposed sample vs. unperturbed cells). The hypothesis behind this method is that a drug may have a therapeutic effect on a disease if their differential gene expression profiles are opposite.[7] In the past, this method has been successfully applied to identify both known and previously unrecognized treatments for inflammatory bowel disease,[8] dermatomyositis,[9] and liver cancer.[10] In addition, it has been used to identify therapeutics for preterm birth[11] and COVID-19,[12] indicating the potential applications of drug repurposing to reproductive health.

In the past, transcriptomics work in endometriosis has allowed us to characterize the unique environment of endometrial lesions, which includes distinctive perivascular mural cells that promote angiogenesis and immune cell migration[13]; analyze patterns in gene expression between healthy controls and endometriosis patients, taking into account age, disease stage, menstrual cycle phase, and/or other clinical factors[14,15]; apply computational approaches to identify the individual contributions of cell subtypes to the overall endometriosis phenotype[15]; and identify specific subtypes of cells that are only enriched in control or diseased tissue — proliferating uterine natural killer cells are uniquely enriched in healthy samples, and endometrial stromal cells are enriched in disease samples.[16] Transcriptomic profiling and analysis have allowed us to better understand the mechanism underlying endometriosis, and the greater availability of public datasets creates opportunities for drug repurposing.

In this study, a computational drug repurposing pipeline was applied to endometrial gene expression data in the setting of endometriosis and controls in order to identify potential therapeutics from existing drugs based on expression reversal. Moreover, we used an established rat model to validate the NSAID fenoprofen, our top drug candidate, as a potential endometriosis therapeutic.

RESULTS

Figure 1 provides an overview of the study. We leverage a publicly available gene expression dataset of 105 endometriosis and healthy control samples which are further stratified into several comparisons: Control vs. all phases and all stages, Control vs. all stages stratified by menstrual phase, Control vs. Stage I-II or Stage III-IV for all menstrual phases. The resulting signatures are then queried against a collection of human cell lines treated with a number of therapeutic compounds for drugs that significantly reverse the disease associated expression. A candidate of interest is further validated in an animal model.

Computational identification of drug repurposing candidates

The gene expression data were derived from 105 samples collected from women with minimal to mild (stage I–II) or more advanced (stage III-IV) endometriosis, and those without uterine or pelvic pathology (control) in proliferative, early secretory, and mid-secretory phases of the menstrual cycle. General cohort characteristics are shown in Table 1. Differential expression analysis as well as pathway and cell type enrichment analysis has been carried out and described in our prior work in greater detail.[17] The numbers of significant differentially expressed genes from unstratified, stage-stratified (i.e., stage I–II or stage III-IV), and phase-stratified (i.e., PE, MSE, or ESE) comparisons of patients with endometriosis to patients in the control cohort are represented in Table S1, with the specific differentially expressed genes represented in Table S2. We expected that gene expression in endometrial tissue would be affected by menstrual phase as well as severity or stage of disease. In order to identify counteracting drugs for the broadest possible population with endometriosis we identified DEG common to all menstrual phases and at all stages of disease. Out of the 3,131 unique genes differentially expressed by at least one endometriosis signature and represented in the CMap database, only 308, or 9.8%, were common across all six signatures including several well known associations such as FOSB, FOS, JUNB, and EGR1 (Figure 2). Pathway analysis, conducted with GSEA targeting MSigDB's Hallmark Pathways and described in our prior work,[17] confirmed the general concordance across stratifications and also showed that many immune pathways such as interferon alpha and gamma responses, TGF-beta and IL-2 STAT5 signaling, and complement pathways were upregulated in endometriosis consistently across most menstrual phase and disease stage stratifications and that TNF alpha signaling and allograft rejection, were significantly downregulated in disease across most stratifications.

By analyzing via our drug repositioning pipeline, the unstratified and stratified differential gene expression signatures with the drug signatures from the CMap dataset, 236–289 drug candidates were determined per signature and 299 unique drugs that significantly reversed the expression profiles of the disease (Table S3). A heatmap of each endometriosis gene expression signature and the gene expression signature for its respective top 24 drug candidates is shown in Figures S1–S6. Similarities across the six signatures were more pronounced for drug candidates than for differentially expressed genes: 221 out of 299 drugs, or 73.9%, were common to every signature (Figure 3A, and Table S3). Of the 221 drugs common across all six signatures, many returned high reversal scores across the board, suggesting that these compounds could be used to treat different stages of endometriosis and across every menstrual cycle phase. As there was consistency in the majority of the drug

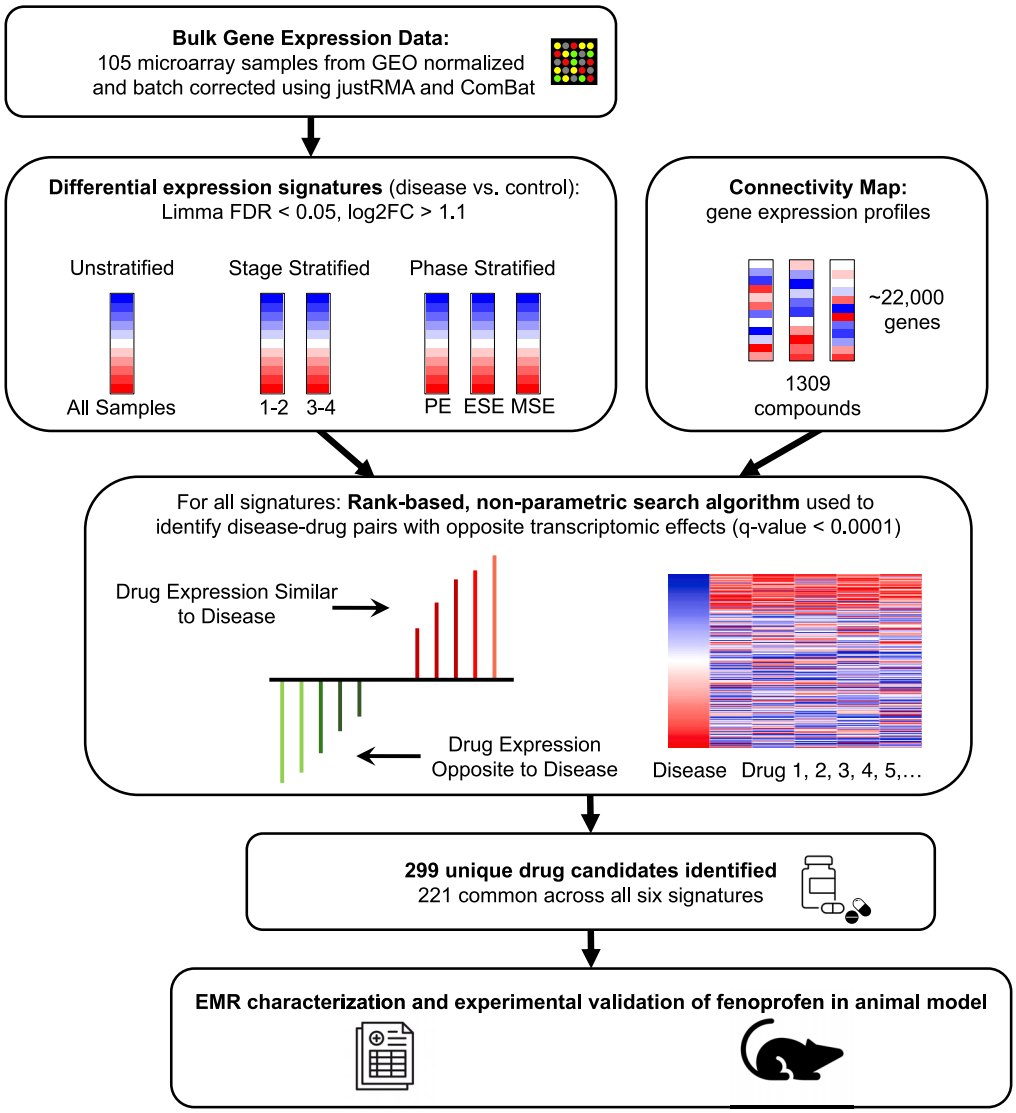


Figure 1. Overview workflow

Experimental overview showing from where and how endometrial tissue transcriptome microarray data were processed and analyzed, then unstratified and stratified differential gene expression signatures were determined using a False Discovery Rate (FDR) cutoff of 0.05 and a log2 fold change (log2FC) cutoff of 1.1, drug repositioning candidates identified through the use of drug expression data from CMap and the computational drug repositioning pipeline, and fenoprofen selected and (1) characterized in electronic medical records and (2) validated through an animal model study.

candidates from the unstratified and stratified signatures as demonstrated by an overlap of 221 candidates (Figure 3A), we moved forward with additional exploration of the top hits based on predictions from the unstratified endometriosis signature.

Several of the drug candidates identified are from classes of medications used to treat endometriosis. Levonorgestrel, among the top 10 drug candidates, is a progestin recommended for treating endometriosis.[18] Also among the identified drug candidates are NSAIDs such as

Table 1. Cohort statistics

	Control	Stages I-II	Stages III-IV	Total
PE	20	10	17	47
ESE	6	6	12	24
MSE	8	8	18	34
Total	34	24	47	105

Numbers in cohort by disease severity (Control, Stages I-II, and Stages III-IV) and by cycle-phase (proliferative (PE), early secretory (ESE), and mid-secretory (MSE)).

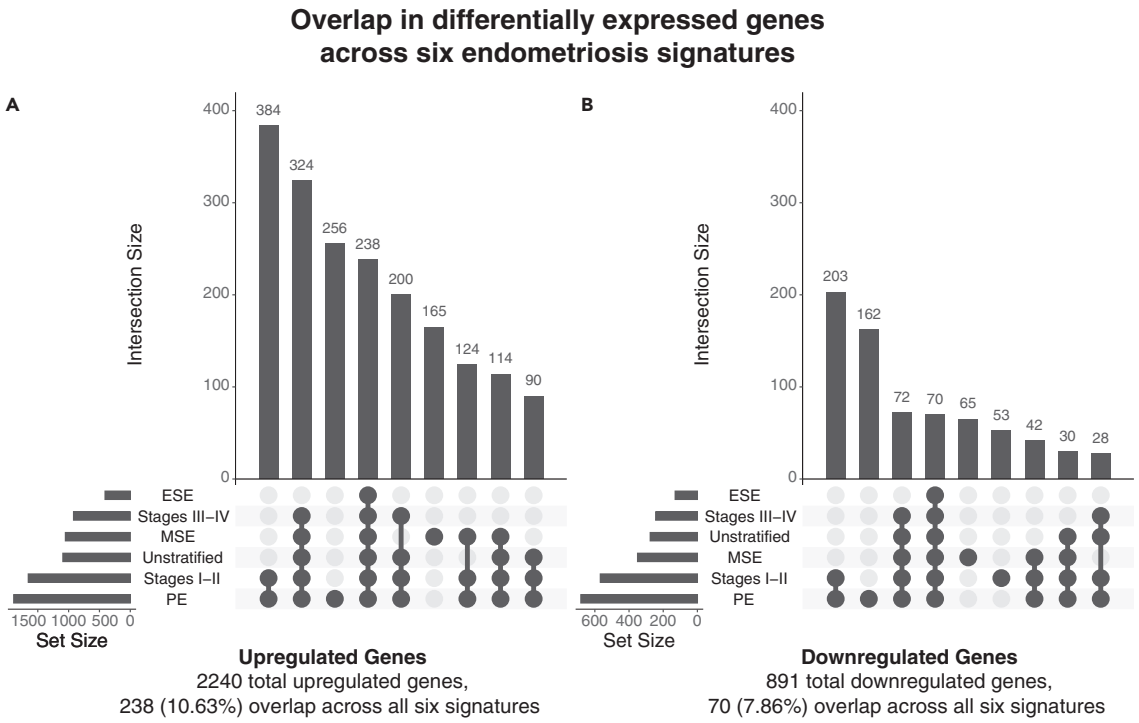


Figure 2. Overlaps in upregulated and downregulated differentially expressed genes between all six endometriosis signatures

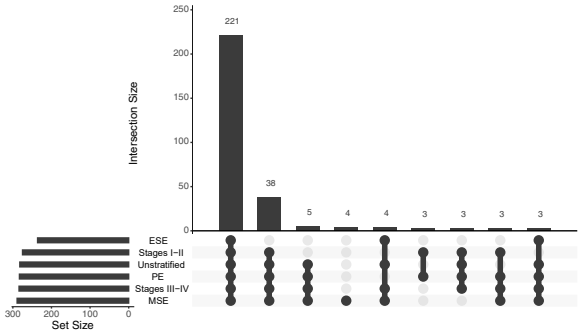
(A) Upregulated genes in all signatures and (B) downregulated genes in all signatures meeting differential expression cutoffs of False Discovery Rate <0.05 and log2 fold change >1.1.

acetylsalicylic acid (commonly known as aspirin), mefenamic acid, indomethacin, naproxen, and diclofenac, which are frequently recommended to alleviate pain and inflammation in dysmenorrhea patients. The NSAID ibuprofen, its isomer dexibuprofen, and the COX-2 selective inhibitor NSAIDs celecoxib, rofecoxib, and valdecoxib are not among our predicted therapeutic candidates since ibuprofen is not represented in CMAP, and dexibuprofen as well as the COX-2 inhibitors are represented in CMAP but are filtered out during pre-processing due to profile inconsistencies.[10] Available descriptions from DrugBank for the drug candidates are in Table S4. A heatmap of the top 20 drug candidates for the unstratified signature and their reversal scores for the six endometriosis signatures is shown in Figure 3B and demonstrates consistency of predictions across the signatures.

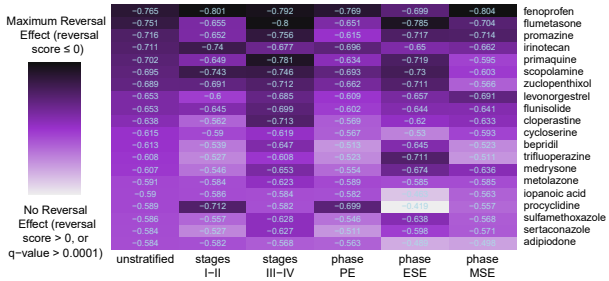
Using the DrugBank database,[19] we were able to identify several proteins targeted by our top 20 drug candidates, including 13 that were targeted by two or more of identified drugs. These interactions were used to construct a network, which can be used to visualize the unique and shared interactions between the top 20 drug candidates and their protein targets (Figure 3C). Out of the proteins displayed in the network, several were found to have a link to endometriosis. Peroxisome proliferator activated receptors gamma and alpha (PPARG and PPARA), which are commonly targeted by NSAID drugs including fenoprofen, can impede the growth of endometrial tissue when activated.[20,21] Prostaglandin-endoperoxidase synthase 2 (PTGS2) gene expression has been found to be significantly increased in ectopic and eutopic endometrium of women with endometriosis compared to women without this condition.[22,23] Moreover, among endometriosis patients, PTGS2 expression in eutopic endometrium has been shown to be significantly greater in women with higher pain scores for dysmenorrhea.[24] Dopamine receptor type-2 (DRD2) polymorphisms have been identified in patients with endometriosis, and treatment with DRD2 agonists has been associated with the disappearance or decrease in size of peritoneal endometriotic lesions.[25,26] Increased gene expression of steroid 5 alpha-reductase 1 (SRD5A1) has been found in ovarian endometriosis compared to normal endometrium.[27] In addition, the nuclear receptor proteins NR3C1 (nuclear receptor subfamily 3 group C member 1), AR (androgen receptor), PR (progesterone receptor), and ESR1 (estrogen receptor 1) are expressed in endometrial cells.[28,29]

Using the SPOKE database,[30] we were able to identify a subnetwork of our top 20 drug candidates and their neighbor diseases for which the drugs have FDA approval to treat or are in Phase 3 clinical trials, and to visualize the unique and shared interactions between the top drug candidates and these diseases (Figure 3D). Among the many conditions that one or more of the top drugs may treat, several conditions involved the immune system/inflammation (e.g., rheumatoid arthritis, uveitis, allergic conjunctivitis, rhinitis, asthma), neoplasms (e.g., tubular adenocarcinoma, granular cell carcinoma), infectious diseases (urinary tract infection, bacterial infection, pneumocystosis), cardiovascular conditions (e.g., cardiovascular disease, hypertension), and psychiatric conditions (schizophrenia, schizophreniform disorder, and schizoaffective disorder). Seven conditions were targeted by two or more of the top drugs: uveitis, allergic conjunctivitis, pneumocystosis, cardiovascular disease, schizophrenia, schizophreniform disorder, and schizoaffective disorder. Endometriosis is not classified as an autoimmune disease,

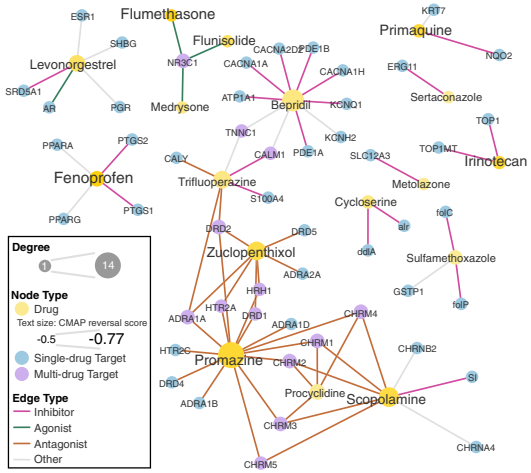
A **Overlap in drugs across six endometriosis signatures (sets with ≥ 3 drugs shown)**



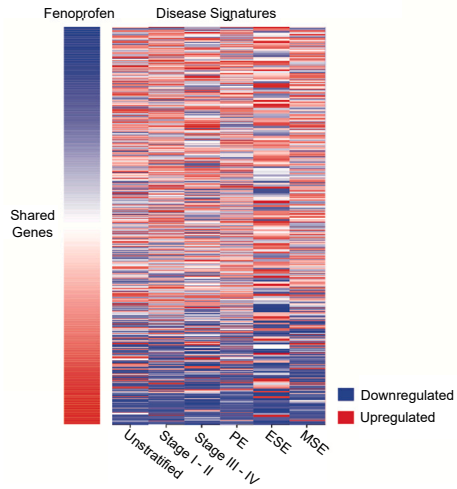
B **Reversal Scores for Top 20 Drug Candidates Across All Endometriosis Signatures**



C **Protein Targets for Top 20 Drug Candidates**



E **Fenoprofen Drug Signature vs Disease Signatures**



D **Disease Targets for Top 20 Drug Candidates**

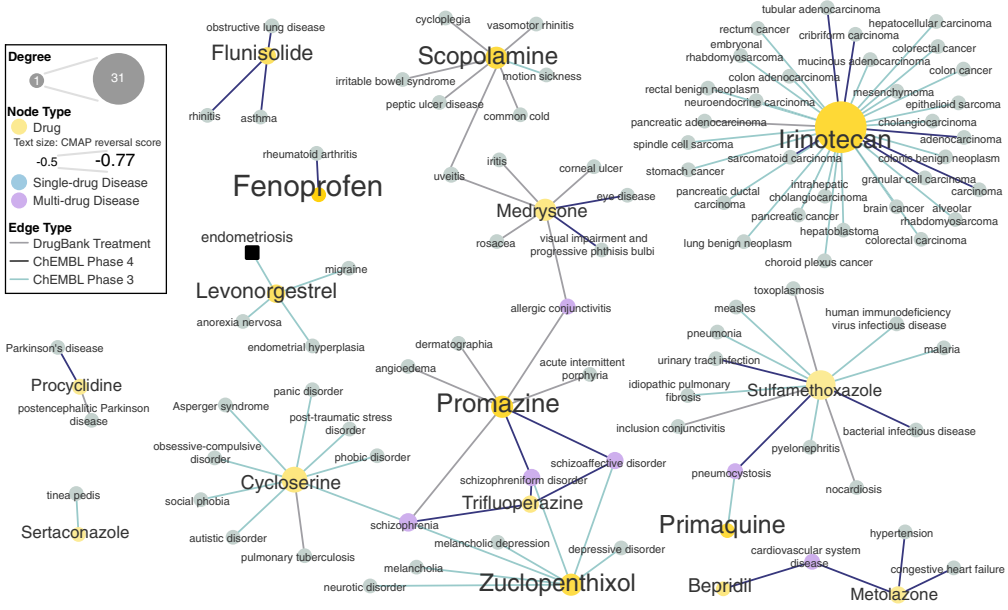


Figure 3. Prediction results

(A) UpSet plot showing overlap in drug candidates across signatures.

(B) Heatmap showing reversal scores for top 20 drugs across signatures.

(C) Drug target network for top 20 drug candidates (data from DrugBank).

(D) Drug disease network for top 20 drug candidates (data from SPOKE).

(E) Heatmap showing fenoprofen drug signature vs. all endometriosis disease signatures.

but studies have found an association between endometriosis and several autoimmune diseases, including rheumatoid arthritis,[31,32] as well as between endometriosis and allergy conditions like allergic conjunctivitis, asthma, and rhinitis.[32] Moreover, associations between endometriosis and infections[33,34] as well as between endometriosis and cancers[33,35–37] have been found. There has been increasing evidence of a relationship between endometriosis and cardiovascular disease, a condition associated with two or more of our top drug candidates, where proposed pathophysiological mechanisms shared by these two diseases include pro-inflammatory, pro-angiogenic, and aberrant immune-endocrine function.[38] While findings from a longitudinal cohort study revealed a high degree of comorbidity between endometriosis and psychiatric disorders including depression, anxiety, and affective psychotic disorders, non-affective psychotic disorders like schizophrenia were not found to be associated with endometriosis[39]; however, top drug candidates promazine and trifluoperazine are phenothiazine antipsychotics, a class of medications found to have potential angiogenesis-reducing and anti-endometrial cancer properties.[40,41]

Through leveraging this approach, fenoprofen, an NSAID commonly used to treat pain and arthritis, was identified as the top drug candidate for the unstratified signature and among the top seven drug candidates for the stratified signatures. When visualizing the gene regulation of the six input endometriosis signatures and fenoprofen, the overall reversal pattern can be observed (Figure 3E). As fenoprofen had the highest reversal score of our drug candidates and belongs to a gold standard treatment category of drugs for endometriosis, our validation efforts herein were focused on this medication.

Animal model validation

To validate the in-silico findings, we used an established animal model of endometriosis that produces vaginal hyperalgesia, a surrogate marker for endometriosis-related pain. In this model, uterine pieces are autotransplanted onto mesenteric abdominal arteries. The uterine transplants develop over a period of weeks into cyst-like structures with characteristics similar to lesions in women with endometriosis.[42] Vaginal hyperalgesia, or an increase in vaginal nociception, develops and stabilizes by ten weeks in this model.[43] In this model, the primary endpoint is the volume of intra-vaginal water within a latex balloon necessary to induce an escape response consistently by a rat due to discomfort. Vaginal hyperalgesia is noted as a decreased volume required to consistently induce the response in a rat. If a therapy is effective at reducing vaginal hyperalgesia, our expectation would be a higher volume required to consistently induce the escape response in a rat. In the context of this study, vaginal nociception was assessed as an escape response to a noxious stimulus – a water filled balloon. Each rat had a balloon placed in its vaginal canal that is inflated 24 times per testing session or "run" – 8 volumes of water (0.01, 0.15, 0.3, 0.4, 0.55, 0.7, 0.8, and 0.9 mL), with each volume delivered 3 times at random. An escape response observed all 3 times (100%) was the maximum per run for a rat. The Median Escape Response (%) is the median proportion of times an escape is observed at a specific volume for all the runs during a test period for a group of rats. We compared four groups of rats: (A) with endometriosis surgery and fenoprofen treatment ("FEN"), (B) with endometriosis surgery and ibuprofen treatment (positive control) ("IBU"), (C) without endometriosis surgery and without treatment (negative control) ("CNS"), and (D) with endometriosis surgery but without treatment (negative control) ("CNT"). In all four groups, vaginal nociception was assessed and data compared over three chronological testing periods: (1) an initial baseline period of eight weeks (2) a post-endo or middle-testing period of ten weeks, and (3) a post-treatment or late-testing period of four weeks.

Responses among fenoprofen (30 mg/kg/day, p.o.) treated animals (n = 6) were significantly increased during the post-endo surgery period compared to the baseline period, when volumes of 0.15, 0.3, 0.4, 0.55, 0.7, and 0.8 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4A, and Table S5). During the post-treatment period, escape responses were significantly decreased compared to the post-endo surgery period when volumes of 0.15, 0.3, 0.4, 0.55, 0.7, and 0.8 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4A, and Table S5). No statistically significant difference was found in the escape responses between the baseline period and the post-treatment period for any volume of water delivered to the fenoprofen treated subjects (Mann Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4A, and Table S5).

Similarly, among ibuprofen (30 mg/kg/day, p.o.) treated animals (n = 6), escape responses were significantly increased during the post-endo surgery period compared to the baseline period, when volumes of 0.15, 0.3, 0.4, 0.55, 0.7, and 0.8 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4B, and Table S6). During the post-treatment period, escape responses were significantly decreased compared to the post-endo surgery period, when volumes of 0.15, 0.3, 0.4, 0.55, 0.7, and 0.8 mL of water were delivered (Mann Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4B, and Table S6). No statistically significant difference was found in the escape responses between the baseline period and the post-treatment period for any volume of water delivered to the Ibuprofen treated subjects (Mann–Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4B, and Table S6).

Among animals that received neither endo surgery nor treatment (n = 6), no statistically significant difference was found in the escape responses between the baseline and post-endo surgery periods, the post-endo surgery and post-treatment periods, or the baseline and post-treatment periods when any volume of water was delivered (Mann–Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figures 4C, and Table S7).

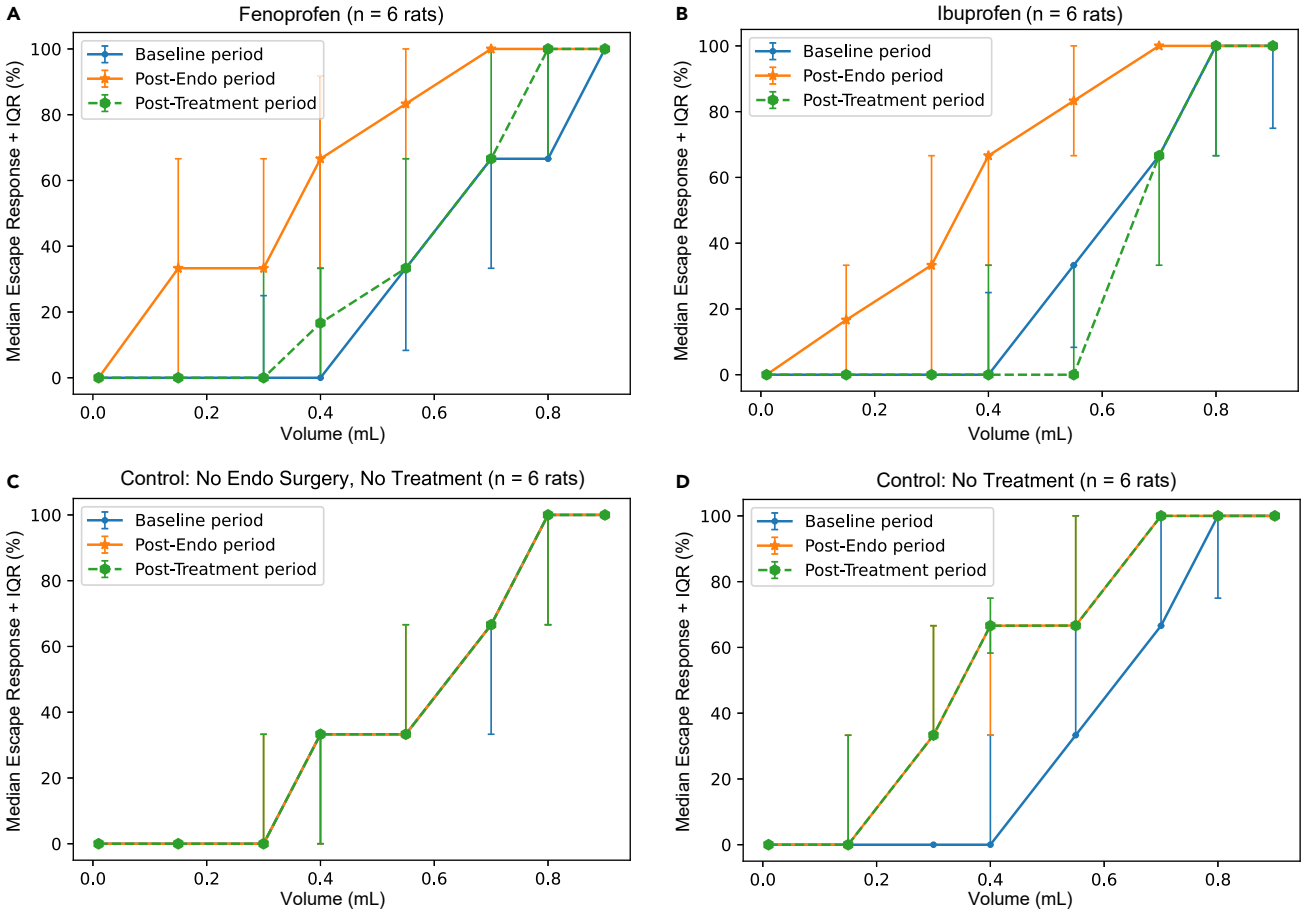


Figure 4. Animal model escape response

Animal model median escape response (%) with interquartile range (IQR; error bars) for each delivered volume during the baseline, post-endo surgery, and post-treatment periods with (A) Fenoprofen treatment (n = 6 rats), (B) Ibuprofen treatment (positive control) (n = 6 rats), (C) No endo surgery and no treatment (negative control) (n = 6 rats), and (D) No treatment (negative control) (n = 6 rats). Volume (mL) (x axis) is the volume of water delivered (0.01, 0.15,0.30, 0.40, 0.55, 0.70, 0.80, and 0.90 mL) to a rat's vaginal canal (via balloon). All "volumes" (8 volumes: 7 volumes and 1 sham) are delivered randomly 3 times each in a 1-h testing session or "run", with an escape response observed all 3 times (100%) as the maximum per run for a rat. The Median Escape Response (%) (y axis) is the median proportion of times an escape is observed at a specific volume for all the runs during a particular period (i.e., baseline, post-endo, or post-treatment) for the group of rats.

Among animals that received endo surgery but no treatment (n = 6), escape responses were significantly increased during the post-endo surgery period compared to the baseline period, when volumes of 0.15, 0.3, 0.4, 0.55, and 0.7 mL of water were delivered (Mann–Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4D, and Table S8). During the post-treatment period, escape responses were also significantly increased compared to the baseline period when volumes of 0.3, 0.4, and 0.55 mL of water were delivered (Mann–Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4D, and Table S8). No statistically significant difference was found in the escape responses between the post-endo surgery period and the post-treatment period for any volume of water delivered to these subjects (Mann–Whitney U test, Bonferroni-corrected p value threshold of 0.05. Figure 4D, and Table S8).

Figure S7 and Table S9 represent the findings from the comparison of the escape responses of the four groups (a) CNS vs. CNT, (b) CNS vs. FEN, (c) CNS vs. IBU, (d) CNT vs. FEN, (e) CNT vs. IVU, and (f) FEN vs. IBU for each of the three condition periods (the baseline period, the post-endo surgery period, and post-treatment period) at each volume of water (0.01, 0.15, 0.3, 0.4, 0.55, 0.7, 0.8, and 0.9 mL) delivered to the balloon placed within the vagina of the rats. In summary, for the Baseline period, there were no significant differences found in the escape responses between the four groups at any volume. For the post-endo period, at volumes 0.3, 0.4, 0.55, and 0.7 mL, there were significant differences found between the control no surgery group and the other three groups; however, these differences between the control no surgery vs. other groups were less/not significant at the lower (0.01, 0.15 mL) and higher (0.8, 0.9 mL) water volumes. For the post-treatment period, at volumes 0.3, 0.4, 0.55, and 0.7 mL, there were significant differences found between the control no treatment group and the other three groups; however, these differences between the control no treatment vs. other groups were less/not significant at the lower (0.01, 0.15 mL) and higher (0.8, 0.9 mL) water volumes. In comparing the fenoprofen treated and ibuprofen treated groups across the three periods,

Table 2. Prevalence of NSAID prescriptions

Institution	Patients with endometriosis, chronic pelvic pain, or dysmenorrhea	Patients with endometriosis, chronic pelvic pain, or dysmenorrhea and prescribed any NSAID (%)	Patients with endometriosis, chronic pelvic pain, or dysmenorrhea and prescribed fenoprofen (%)
UCSF	12476	7752 (62.14%)	1 (0.008%)
UCD	10040	6163 (61.38%)	0 (0%)
UCI	5694	3545 (62.26%)	0 (0%)
UCLA	23017	13041 (56.66%)	3 (0.013%)
UCSD	10079	6042 (59.95%)	1 (0.010%)
Total	61306	36543 (59.61%)	5 (0.008%)

Patients with endometriosis, chronic pelvic pain, or dysmenorrhea and prescribed (a) any NSAID and (b) fenoprofen at five UC Health Care Institutions (UC San Francisco (UCSF), UC Davis (UCD), UC Irvine (UCI), UC Los Angeles (UCLA), UC San Diego (UCSD)).

there were no significant differences found in escape responses for the majority of volumes except at 0.55 mL for the post-treatment period where the escape response for the ibuprofen-treated group (median = 0) was lower than for the fenoprofen-treated group (median = 33%) (MWU test, $p \leq 0.05$).

Electronic medical record analysis

From the analysis of electronic medical records (EMR) across five University of California (UC) healthcare institutions (UC San Francisco (UCSF), UC Davis, UC Irvine, UC Los Angeles, and UC San Diego), there were a total of 61,306 patients with endometriosis, chronic pelvic pain, or dysmenorrhea, among whom 36,543 (59.61%) had a prescription for an NSAID and 5 (0.008%) had a prescription for fenoprofen (Table 2). For the individual UC healthcare institutions, among the 12,476 patients at UCSF with endometriosis, chronic pelvic pain, or dysmenorrhea, 7,752 (62.14%) had a prescription for an NSAID and 1 (0.008%) had a prescription for fenoprofen; among the 10,040 patients at UC Davis with endometriosis, chronic pelvic pain, or dysmenorrhea, 6163 (61.38%) had a prescription for an NSAID and 0 (0.000%) had a prescription for fenoprofen; among the 5,694 patients at UC Irvine with endometriosis, chronic pelvic pain, or dysmenorrhea, 3,545 (62.26%) had a prescription for an NSAID and 0 (0.000%) had a prescription for fenoprofen; among the 23017 patients at UC Los Angeles with endometriosis, chronic pelvic pain, or dysmenorrhea, 13,041 (56.66%) had a prescription for an NSAID and 3 (0.013%) had a prescription for fenoprofen; and among the 10,079 patients at UC San Diego with endometriosis, chronic pelvic pain, or dysmenorrhea, 6,042 (59.95%) had a prescription for an NSAID and 1 (0.010%) had a prescription for fenoprofen (Table 2).

DISCUSSION

Endometriosis is an estrogen-dependent inflammatory disorder, with both local (pelvic) and systemic components that commonly contribute to pelvic pain and infertility.[44–46] Therapies for pain include surgical resection of disease and/or medical approaches mostly aimed at reducing ovarian estrogen action or production. Unfortunately, ~50% of patients need repeat surgery within 5 years or recurrent symptoms, and medical therapies are either ineffective or promote intolerable side effects that limit their long-term use.[1,4] Recent FDA approval of new drugs for endometriosis (e.g., GnRH antagonists Elagolix and Myfembree) brings hope for those who suffer from this disease[47,48]; however, off-target effects (e.g., bone density) await long-term, post-marketing studies. Thus, there is a pressing need for novel drug discovery to improve patient symptoms and quality of life. Our study identified several existing drugs with potential therapeutic applications to endometriosis using a transcriptomics-based computational drug repurposing approach. The differential gene expression profiles for endometriosis that were for the overall unstratified dataset as well as, for sensitivity analysis, stratified by disease stage and menstrual cycle phase were compared with the profiles of several small molecule compounds tested on human cell lines, yielding 299 unique drug candidates with significant (q-value <0.0001) reversal effects. We found that therapeutic predictions were relatively consistent across the overall and stage- and cycle phase stratified analyses with 221 shared predictions.

Pain of endometriosis can occur at any ASRM disease stage/severity or any cycle-phase,[1] hence our team sought to find drugs that were identified by our drug-repositioning pipeline regardless of the ASRM disease stage or cycle-phase that the eutopic endometrial data represented (i.e., stages I & II, stages III & IV, cycle-phase ESE, cycle-phase MSE, or cycle-phase PSE). The most prevalent theory of pelvic endometriosis pathogenesis is that it is derives from eutopic endometrium shed at the time of menses that travels retrograde through the fallopian tubes, arriving in the pelvis, eliciting an inflammatory response with neuroangiogenesis and promoting lesion growth, fibrosis, and pain.[45] Strong data to support the origins of the disease derive from observations that endometriosis lesions and eutopic endometrium share specific cancer driver mutations[49] and display abnormal progesterone signaling and a pro-inflammatory environment,[50] although the ectopic (disease) tissue has a more extreme phenotype. In the current study, we analyzed transcriptomic data of the eutopic endometrium from individuals with versus without endometriosis in an overall unstratified manner as well as stratified by ASRM disease stage and menstrual cycle phase for possible drug candidates to reverse pathways therein relevant across disease stages and cycle phases to the disease process and symptoms.

When categorized by drug class/ATC code, two prominent categories for the predicted therapeutics were anti-inflammatory drugs and sex hormones; drugs from both categories have extensively been used to treat endometriosis.[3] Several drugs that the pipeline returned are current

gold standard treatments, such as levonorgestrel, mefenamic acid, acetylsalicylic acid (aspirin), and naproxen; others were unexplored candidates.

From the drugs identified by the computational drug repurposing approach, we chose fenoprofen for further validation since it returned the highest reversal score and belongs to a class of drugs (NSAIDs) that is a current first-line treatment for endometriosis. Fenoprofen is a medication available by prescription only and has been in clinical use since this drug was approved by the FDA in 1976[51] and is indicated for the relief of mild to moderate pain in adults and, in particular, relief of signs and symptoms of rheumatoid arthritis and osteoarthritis.[52] In our analysis of the electronic medical records across five University of California healthcare institutions, we found that while NSAIDs have been commonly prescribed (56.66%–62.26%) for patients with endometriosis, chronic pelvic pain, or dysmenorrhea diagnosis, the NSAID fenoprofen was prescribed for the minority (0%–0.013%) of patients with these conditions.

We tested the NSAID fenoprofen in a rat model of endometriosis that displays vaginal hyperalgesia, a surrogate marker for endometriosis-related pain. We determined that oral treatment with fenoprofen significantly alleviated endometriosis-associated vaginal hyperalgesia, similar to oral ibuprofen treatment, and there were mostly no significant differences in the escape responses comparing these two NSAIDs. In endometriosis rats with no treatment, vaginal hyperalgesia was maintained, which confirmed that the alleviation of hyperalgesia observed in the treatment groups was not due to additional vaginal nociceptive testing post-endometriosis. In rats with no endometriosis and no treatment, no significant changes in vaginal nociception occurred, which suggests that any observed changes in the other groups were not due to lengthy vaginal nociceptive testing alone. Overall, these findings support fenoprofen as a therapeutic that could be used more commonly for endometriosis-associated pain.

NSAIDs prevent or reduce production of prostaglandins, which in turn can help relieve pain from endometriosis. Moreover, NSAIDs have long been known to have immunomodulatory properties,[53] and pathway analysis from our previous work demonstrated that many immune pathways are among those associated with endometriosis, including Interferon alpha and gamma responses, TGF-beta and IL-2 STAT5 signaling, complement pathways which were upregulated, and TNF alpha signaling and allograft rejection which were downregulated.[15] While NSAIDs are a commonly prescribed, first-line treatment of endometriosis, there is currently limited evidence to support the effectiveness of any NSAID over another for endometriosis pain relief.[54] Our findings suggest that the NSAID fenoprofen, currently infrequently prescribed for endometriosis, may be an effective treatment for individuals with this condition, although this would need further validation in patient cohorts. There are many different NSAIDs, each with subtle but important differences in their effect on host physiology. Work like this could eventually guide clinical care from starting any NSAID toward the use of NSAIDs specifically more effective for endometriosis.

Our drug target network analysis for the top drug candidates shows that PPARG and PPARA, which impede the growth of endometrial tissue when activated, are both targeted by fenoprofen.[20,21] In addition, our network analysis showed that fenoprofen targets the enzymes PTGS1 and PTGS2 (i.e., COX1 and COX2, respectively). PTGS1 and PTGS2 have been shown to be inhibited to varying degrees in blood and gastric tissue by the available NSAIDs,[55] but any differences in the degree of inhibition of PTGS1 and PTGS2 in endometrial tissue by fenoprofen and other NSAIDs have not yet been demonstrated. PTGS1 is a constitutively expressed enzyme and involved in maintaining cell homeostasis.[56] In contrast, PTGS2 is an enzyme uncommonly expressed normally, but is induced during inflammation as well as cell proliferation and differentiation.[22] Significantly increased gene expression of PTGS2 has been found in the ectopic and eutopic endometrium of women with endometriosis compared to women without this condition.[22,23] Furthermore, PTGS2 expression in the eutopic endometrium of women with endometriosis has been shown to be significantly higher in those with more severe dysmenorrhea.[24] Targeting key factors involved in endometriosis pathogenesis and symptomatology may contribute to the effectiveness of fenoprofen in treating endometriosis.

Our drug disease network analysis for the top drug candidates reveals conditions associated with inflammation and the immune system, the cardiovascular system, neoplasms, infectious diseases, and psychiatric conditions. As recent research supports, endometriosis is a systemic disorder that transcends the reproductive organs and can more broadly affect mood, metabolism, autoimmune disorders, cancer risk, and the cardiovascular system.[57–59] Rheumatoid arthritis, a chronic autoimmune disease characterized by joint inflammation and for which our top drug candidate fenoprofen is an FDA-approved treatment, has been found to be significantly associated with endometriosis in recent large case-control and cohort studies[32,60,61] and efforts to explore features that may be shared between these diseases and understand associations on a molecular level have been made.[62–64]

To summarize, we applied a computational drug repurposing pipeline to identify potential therapeutics for endometriosis-related pain. The pipeline returned many known treatments as well as uncommon and previously unrecognized candidates. As a pilot and proof of principle work, we tested the identified therapeutic candidate fenoprofen, an NSAID infrequently prescribed for endometriosis, chronic pelvic pain, or dysmenorrhea, in an established rat model of endometriosis. We determined that fenoprofen successfully alleviated endometriosis-associated vaginal hyperalgesia, a surrogate marker for endometriosis-related pain. These findings validate fenoprofen as a therapeutic that could be utilized more frequently to treat endometriosis and suggest the utility of future investigation into additional drug candidates as well as incorporation of single cell expression profiles to extend this work to predicting combination therapies.

Limitations of the study

Our study has several limitations. This study is meant to demonstrate the feasibility of the approach. While we use molecular (expression) profiles for prediction of therapeutics, the presented approach does not allow for understanding of the mechanism of action of the drug at the molecular level. It is possible that the effect that we observe of fenoprofen for endometriosis is not only due to its known effects on prostaglandin synthesis by blocking cyclooxygenase but also its off-target effects. Additional experiments are needed as next steps to further understand the implications of the findings on disease burden and compound mechanism of action. The endometriosis signatures

were generated from bulk gene data that included 105 microarray samples; they could be made more robust by incorporating additional public expression datasets. Single-cell data could also be used to investigate the potential effects of drug candidates on specific types of endometrial cells and for combination therapy predictions. Although here we used transcriptomics from endometrium rather than endometriosis lesions, future work should explore signatures of the lesions themselves to query the drug data for therapeutic discovery. Drugs like ibuprofen and GnRH antagonists that lack representation in the CMAP data used by the drug repurposing pipeline would not be identified by the pipeline, nor would drugs that are represented in CMAP but filtered out during pre-processing due to profile inconsistencies,[10] such as the COX-2 selective inhibitor NSAIDs. The nature of the drug repurposing pipeline prioritizes drugs that have a high reversal effect on the disease signature; it does not take into account whether transcriptional effects are limited solely to genes that the disease also affects. A drug that causes wide-ranging gene changes—including reversal to the gene changes caused by endometriosis—may present in the list of identified drugs, and may be therapeutic. However, unrelated gene changes could cause undesirable side effects, and depending on specificity and severity of side effects, these drugs may have limited applicability clinically. Moreover, in our study, we did not assess the effects of fenoprofen on disease burden. Fenoprofen currently requires a prescription, can be among the higher cost NSAIDS, and carries as all NSAIDs do an FDA warning for serious cardiovascular and gastrointestinal side effects.[65,66] Any immediate and long-term consequences of fenoprofen use in patients with endometriosis would need to be monitored. Out of all the drug candidates across every endometriosis signature, many were antipsychotics and other drugs that affect a wide range of genes. In addition, while we and others have leveraged the CMap dataset extensively for therapeutic discovery for a number of non-cancer indications,[8,9,11,12] the compounds from the CMap dataset were tested on cancer cell lines; the drugs' effects on endometrial tissue would be far better determiners of their potential applications to endometriosis. Unfortunately, such a large number of compounds have not been yet tested on relevant endometrial tissues; however, as newer datasets become available those will be incorporated into our future drug discovery efforts for endometriosis. To explore drug-protein interactions, we leveraged the DrugBank database which contains extensive data on drug targets but potentially not off-target interactions between drugs and proteins and other molecules. Further *in vitro* work would be needed to investigate and validate the interactions of our top therapeutic candidates. A further limitation of our study is that we assessed fenoprofen as an endometriosis therapeutic in a rodent model. Notably, disease burden in animal models and women assessed by the ASRM staging system reveals that disease burden does not correlate well with pain extent or location, except some deep infiltrating disease.[67,68] This is a complicated issue in the regulatory domain, as the FDA requires two co-primary endpoints of dysmenorrhea and non-menstrual pelvic pain (NMPP) in endometriosis-related pain studies, but does not require disease burden assessment. Imaging disease lesions before and after candidate drug trials can be pursued in animal models where, e.g., fluorescent probes enable such. In humans, a second surgery would be required to assess treatment effects on disease burden/size, which is rarely performed for logistical and patient safety and expense of a repeat laparoscopy, especially when pain is the primary outcome. Although our model mimics many disease features of women with endometriosis, rats do not menstruate or develop endometriosis spontaneously. Therefore, menstruating non-human primates could be considered a more appropriate model as they develop endometriosis spontaneously; however, because of their close phylogenetic relationship to humans, these models come with unique ethical considerations[69,70] as well as limiting financial cost. We aimed to obtain data on efficacy of fenoprofen in patients by querying the electronic medical record; however, as we found so few patients on fenoprofen, we did not have sufficient statistical power to carry out this analysis. A clinical trial would be needed to obtain information regarding efficacy of this drug in treating endometriosis.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

D.B., A.B., L.C.G., S.L.M., and M.S. designed the study, experiments, and analytic plan. T.T.O., A.B., D.B., A.S.T., C.L., and S.L.M. carried out data acquisition, processing, and analysis. T.T.O., A.B., D.B., B.L.L., I.K., B.G., D.K.S., J.C.I., L.C.G., S.L.M., and M.S. carried out computational and statistical analysis. T.T.O., A.B., D.B., B.L.L., I.K., B.G., D.K.S., J.C.I., L.C.G., S.L.M., and M.S. interpreted results. E.A., A.G., L.M., L.L., and S.L.M. carried out the validation experiments and analyzed the relevant data. T.T.O., A.B., and S.L.M. wrote the manuscript. All the authors participated in relevant discussions, edited and reviewed the manuscript.

DECLARATION OF INTERESTS

A.B., D.B., T.T.O., L.C.G., and M.S. have a patent related to this work. B.L.L. is working for Nine Square Therapeutics. L.C.G. is a paid consultant to Myovant Sciences, Gensyta Pharma, Celmatix, NextGen Jane, and Chugai Pharmaceutical Co. The remaining authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Molecular Classification of Endometriosis and Disease Stage Using High-Dimensional Genomic Data	Tamaresis et al.[17]	NCBI GEO: GSE51981
Experimental models: Organisms/strains		
Sprague-Dawley rats	Charles River (Wilmington, MA; Raleigh NC facility)	Crl:CD(SD); RRID: RGD_734476
Software and algorithms		
Endometrial Deconvolution code	Bunis et al.[15]	https://github.com/dtm2451/EndometrialDeconvolution
Endometriosis Drug Repurposing. – Differential Gene Expression code	Bunis et al.[15]	https://github.com/dtm2451/EndometriosisDrugRepurposing
Drug repurposing pipeline	Chen et al[10]	https://github.com/Bin-Chen-Lab/HCC_NEN
Other		
University of California Health Data Warehouse (UCHDW)	Center for Data-driven Insights and Innovation (CDI2)	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Marina Sirota (marina.sirota@ucsf.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) Database (series accession number GEO: GSE51981) https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51981 and are publicly accessible. The UCSF EMR database is available to UCSF-affiliated individuals who can contact UCSF's Clinical and Translational Science Institute (CTSI) (ctsi@ucsf.edu) or the UCSF's Information Commons team for more information (Info.Commons@ucsf.edu). UCDDP is only available to UC researchers who have completed analyses in their respective UC first and have provided justification for scaling their analyses across UC health centers.
- Code for transcriptomic data processing associated with the current submission is available at https://github.com/dtm2451/EndometrialDeconvolution and https://github.com/dtm2451/EndometriosisDrugRepurposing, and code for the computational drug repurposing pipeline associated with the current submission is available at https://doi.org/10.1053/j.gastro.2017.02.039.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animal model validation

Subjects and vaginal cytology

Animal subjects were 24 adult virgin female Sprague-Dawley rats obtained from Charles River (Wilmington, MA; Raleigh NC facility). They weighed 175–225 g at the start of the study and were housed individually in a temperature-controlled room (22.2°C) in plastic cages lined with chip bedding, with *ad libitum* access to rat chow and water. They were maintained on a 12-h light/dark cycle, with lights on at 07:00.

Reproductive status was determined by daily vaginal lavage performed ~2h after lights on for all rats.[71] Traditional nomenclature was used for the four estrous stages of proestrus, estrus, metestrus, and diestrus.[71] All rats maintained normal four-day estrous cycles throughout

training and testing. All behavioral training and testing were done ~3–8 h after lights on. The study and procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) as protocol #2021000201. All laboratory animal experimentation adhered to the NIH Guide for the Care and Use of Laboratory Animals.

Experimental groups

There were four groups of rats analyzed: Group FEN: ENDO, fenoprofen (30 mg/kg/day, p.o) (n=6); Group IBU: ENDO, ibuprofen (30 mg/kg/day, p.o.) (positive control) (n=6); Group CNT: ENDO, no treatment (negative control) (n=6); and Group CNS: no ENDO, no treatment (negative control) (n=6). Ibuprofen was selected as our positive control as it is a commonly used analgesic agent for rodents and has been effectively used in pain studies in rodents (e.g. inflammatory pain models).[72–74] Data from all groups were compared between three chronological testing periods: (i) testing period 1: an initial baseline period of 8 weeks, (ii) testing period 2: a post-ENDO or middle-testing period of 10 weeks, and (iii) testing period 3: a post-treatment or late-testing period of 4 weeks. In doing so, each animal serves as its own control (i.e., within-subjects design). Data for three of six rats in control Groups CNS and CNT were retrieved and reanalyzed from an earlier study.[43]

Endometriosis model (ENDO)

Rats were surgically induced with endometriosis based on the model by Vernon and Wilson, and described as follows.[75] Rats in diestrus were anesthetized intraperitoneally with a mixture of ketamine hydrochloride (73 mg/kg) and xylazine (8.8 mg/ kg) and placed on a heating pad to maintain body temperature ~37°C. A midline abdominal incision was made to expose the uterus and a ~1-cm segment of the left uterine horn and associated fat tissue were removed and placed in warm sterile saline. Four pieces of uterine horn (~ 2 mm × 2 mm) were cut from this segment and sewn around alternate cascade mesenteric arteries that supply the caudal small intestine starting from the caecum using 4.0 nylon sutures. After making sure there was no bleeding in the abdominal cavity, the wound was closed in layers. Rats were closely observed during the postsurgical period for potential complications (none occurred). Postoperative recovery was uneventful, and regular estrous cyclicity resumed in all rats within a few days.

Behavioral assessment of vaginal nociception. The behavioral training and testing procedures were based on work by Berkley and performed as follows.[76] Rats were trained to perform an escape response to terminate vaginal distention produced by an inflatable latex balloon. During each testing session, eight different distention volumes were delivered three times each in random order at intervals of ~60 seconds, and percent escape response to each volume was assessed.

Behavioral apparatus and stimulator. The training and testing apparatus was a small rectangular, grill-floored Plexiglas® chamber designed to contain the rat just enough to prevent her from turning around. A hollow tube containing light-emitting diodes and a photosensor extended from the front of the chamber. If the rat extended its nose into this tube, a light beam was broken that terminated the stimulus. In other words, the rat's breaking the light beam constituted an escape response. An opening in the rear of the chamber allowed the catheter (attached to the vaginal stimulator) to be connected to the computer-controlled and automated stimulus-delivery device.

The vaginal stimulator was a small latex balloon (~ 10mm long × 1.5 mm wide when uninflated) tied to a thin catheter with silk suture. Immediately prior to the training or testing session, the uninflated balloon was lubricated with K-Y® jelly and inserted into the mid-vaginal canal, located so that it would not touch the cervix even when inflated. Inflating the balloon with different volumes of water using a computer-controlled pump distended the vaginal canal. The pressure produced by each volume of distention (corrected for compliance characteristics of the balloon) was measured through a small-volume Cobe pressure transducer.

Behavioral training. After the rat was first adapted to the testing chamber by placing her in it for 10 min daily for 3–4 days (and feeding her small amounts of peanut butter on a wood stick), training sessions began in which the trainer pinched the rat's tail with a padded forceps, using its release to shape a required "escape response," which involved the rat extending her head into the tube to interrupt the light beam. Training sessions of 10 pinches delivered at ~1 min-intervals were run 3/week on non-consecutive days. Training was completed (>80% escape behavior) in 4–8 sessions.

The rat was next trained to make identical escape responses to deflate vaginal distention stimuli. These sessions were run 3 times/week on non-consecutive days for a total of 3–5 sessions. Ten large distention volumes (0.80 ml – 1.0 ml, inflation rate 1 ml/s) were delivered for a maximum of 15 s at ~1-min intervals. All rats showed some behavioral response to these stimuli, which allowed the experimenter to use deflation of the vaginal balloon to shape the rat's escape responses. All rats learned the escape response within 2–4 sessions. Once trained, testing sessions began.

Electronic medical records analysis

The study was approved by the University of California, San Francisco, institutional review board (as IRB number 22-37954) and considered secondary research for which consent is not required. Patients with endometriosis, chronic pelvic pain, or dysmenorrhea who were prescribed (a) any NSAID and (b) fenoprofen were identified from the UC Data Discovery Portal's UC-wide deidentified OMOP-based EMR database, which includes clinical data from over 8 million patients from January 1, 2012 to July 30, 2022 at UC San Francisco, UC Davis, UC Irvine, UC Los Angeles, and UC San Diego. During August 2022, patients from these five UC institutions with endometriosis, chronic pelvic pain,

or dysmenorrhea were identified by inclusion criteria of having a self- or provider- identified sex of female with at least one OMOP concept id in the condition_occurrence table for endometriosis (OMOP concept ids 4211992, 37117191, 4072148, 4146995, 4264439, 4182703, 36713393, 4288543, 4307585, 4176409, 4051345, 4058381, 4200841, 4260818, 194420, 4272614, 4132140, 37209400, 197033, 4222798, 4317964, 4127413, 4019817, 139882, 37209399, 199881, 4189364, 36713394, 4276944, 37119080, 194421, 4230333, 46273242, 42536674, 36717630, 433527, 37110261, 37110262, 4224161, 4195507, 37209188, 4034016, 44806162, 37396113, 44806981, 4167725, 42737048, 2109446, 42737049, 2109445, 2109444, 4306918, 4202522, and 4270918), for chronic pelvic pain (OMOP concept ids 4133035, 4034006, and 42534971), or for dysmenorrhea (OMOP concept ids 4137754, 4159586, 4117874, and 194696). Among 4,441,860 female patients in the EMR (mean (SD) age: 45.8 (22.8) years), 60,518 patients were identified with at least code in the condition_occurrence table for endometriosis, chronic pelvic pain, and/or dysmenorrhea (mean (SD) age: 39.8 (13.8) years, Hispanic or Latino: 11,231 (18.6%), Not Hispanic or Latino: 43,450 (71.8%), Unknown ethnicity: 5,837 (9.6%); American Indian or Alaska Native: 277 (0.5%), Asian: 6,634 (11.0%), Black or African American: 4,398 (7.3%), Native Hawaiian or Other Pacific Islander: 349 (0.6%), Other race, multirace, or unknown race: 14,722 (24.3%), White: 31,682 (52.4%).

METHOD DETAILS

Gene expression signature of endometriosis

Microarray-based transcriptional profiling data from eutopic endometrial tissues of women (n=148) classified as with endometriosis ("E")(n=77), with no endometriosis but with uterine/pelvic pathology (e.g., symptomatic uterine fibroids, pelvic organ prolapse, and adenomyosis) ("NE.UPP")(n=37), or with no uterine or pelvic pathology ("NE.NUPP")(n=34) were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) Database (series accession number GEO: GSE51981) and cleaned and batch corrected as done in Bunis et al. (https://github.com/dtm2451/EndometrialDeconvolution/, file: microarray_processing.Rmd).[15]

Computational drug repurposing based on gene expression profiling

To identify potential drug candidates for endometriosis, we used a nonparametric rank-based method based on differential gene expression profiles using the Kolmogorov-Smirnov statistic.[10] The hypothesis is that drugs with opposite transcriptional effects to those observed in endometriosis could potentially have a therapeutic effect in treating endometriosis. On the drug side, CMap was used to obtain gene expression profiles from various cell lines treated with small-molecule drugs. The CMap dataset has gene expression profiles (~22,000 genes) for 1,309 small-molecule drug compounds cultured in up to 5 different cancer cell lines.[77] For endometriosis, differential gene expression signatures were generated as described using six different stratifications: unstratified (all samples), stratified by stage (stages I-II or stages III-IV), and stratified by phase (PE, ESE, or MSE).

To characterize the top 20 drug candidates from the unstratified endometriosis signature and their interactions with proteins in humans, we used data from DrugBank[19] regarding known protein targets of drugs and Cytoscape[78] to visualize these known drug-protein interactions in a network. Furthermore, in order to identify the drug-disease pairs, we performed network analysis using the SPOKE knowledge network,[30] which includes information from the DrugBank and ChEMBL[79] databases, to explore the top 20 drugs from the unstratified endometriosis signature and the diseases they are approved by the FDA to treat and/or for which they are being studied in Phase 3 or Phase 4 clinical trials.

Electronic medical record analysis

Among the patients with at least one of the aforementioned OMOP concept ids for endometriosis, chronic pelvic pain, and/or dysmenorrhea, we determined the number of patients at each UC institution. Furthermore, we identified from the drug_exposure table of the OMOP-based database the number and percentage of these individuals at each UC institution who were ever (a) prescribed at least one of the following NSAIDs: ibuprofen, naproxen, celecoxib, diclofenac, etodolac, indomethacin, piroxicam, sulindac, oxaprozin, meloxicam, diflunisal, ketorolac, meclofenamate, nabumetone, salsalate, and fenoprofen, and (b) prescribed fenoprofen.

Animal model behavioral testing

Testing sessions were run 3 to 4 times/week on non-consecutive days for each rat. Each testing session included a series of 24 computer-controlled escape trials that were run at ~1-min intervals (range 50 – 70 s). Each trial consisted of rapid inflation of the balloon (1 ml/s) to a fixed volume, where it remained until the rat made an escape response or 15 s elapsed, when the balloon rapidly deflated (0.5 ml/s). Eight different distention volumes, including a control volume (0.01 ml), were delivered three times each in random order. The computer recorded stimulus volume, stimulus pressure, and response latency for each trial. The maximum latency of 15 s was considered to be no response. The experimenter was blind to the volumes being delivered to the rat. After the escape trials were run, the rat was given a small amount of peanut butter on a wood stick and removed from the testing chamber.

QUANTIFICATION AND STATISTICAL ANALYSIS

Gene expression signature of endometriosis analysis

Sample metadata were used to classify samples by lab of origin, by disease stages I-II, disease stages III-IV[80] or NUPP control, and by cycle-phases proliferative endometrium (PE), early secretory endometrium (ESE), or mid secretory endometrium (MSE). To minimize potential confounding bias, our analysis excluded NE.UPP samples (n=37), as well as any samples that could not be unambiguously mapped to an aforementioned severity (n=2) or phase-cycle (n=4) classification.[17] Remaining data (total n=105, with E samples (endometriosis samples) (n=71)

and NE.NUPP samples (normal controls) (n=34)) were then normalized with the R package justRMA[81] and batch corrected using the package ComBat[82] to reduce signal associated with lab of origin, while protecting signals associated with the disease stage and the cycle phase. Differential gene expression analyses, using the package limma,[83] were then performed (1) on the overall dataset: unstratified = all normal control and endometriosis samples, and on subsets of the samples for sensitivity analysis: (2) stage-stratified = all normal control samples and endometriosis samples of either (2a) stage I-II or (2b) stage III-IV, and (3) phase-stratified = normal control and endometriosis samples of the given cycle-phase (3a) PE, (3b) ESE, or (3c) MSE. Genes passing cutoffs FDR-adjusted P-value < 0.05 and logFC > 1.1 and that were represented in the Connectivity Map (CMap) dataset from the Broad Institute[47] were considered the significant genes for each signature. Code for differential gene expression analysis is available on GitHub (https://github.com/dtm2451/EndometriosisDrugRepurposing, file: 1_DifferentialExpression_EndometriosisStratifications.Rmd)

Analysis of computational drug repurposing based on gene expression profiling

For each endometriosis disease signature, reversal scores based on the Kolmogorov–Smirnov statistic were calculated for each drug in the CMap dataset based on the relationship between the gene expression profiles for the disease relative to the drug as done in our prior drug repurposing efforts.[8–12] A negative score indicated reverse profiles between the drug and disease signature, where upregulated genes in the disease signature were downregulated in the drug signature and vice versa. A positive score indicated the opposite — similar profiles between the drug and disease signature. For drugs with multiple gene expression profiles from different cell lines or concentrations, we kept the profile with the largest reversal effect, or most negative score. Permutation analysis was carried out to assess significance, and drug candidates with q-values < 0.0001 or reversal scores < 0 (indicating signature reversal) were examined further.

Animal model validation statistical analysis

For each rat group (CNS: without endo surgery, no treatment (negative control) (n=6); CNT: with endo surgery, no treatment (negative control) (n=6); (FEN: with endo surgery, treated with fenoprofen (n=6); IBU: with endo surgery, treated with ibuprofen (positive control) (n=6)), a non-parametric test was performed to compare the escape responses of the 3 testing periods: baseline (24 data points), post-endo surgery (40 data points), and post-treatment (32 data points). Specifically, Mann Whitney U (MWU) tests were performed to compare the escape responses of (a) the baseline period and the post-endo surgery period, (b) the post-endo surgery period and the post-treatment period, and (c) the baseline period and the post-treatment period at each volume of water (0.0, 0.15, 0.3, 0.4, 0.55, 0.7, 0.8, and 0.9 mL) delivered to the balloon placed within the vagina of the rats. Moreover, Mann Whitney U (MWU) tests were performed to compare the escape responses of (a) CNS vs CNT, (b) CNS vs FEN, (c) CNS vs IBU, (d) CNT vs FEN, (e) CNT vs IVU, and (f) FEN vs IBU for each of the three condition periods (the baseline period, the post-endo surgery period, and post-treatment period) at each volume of water (0.0, 0.15, 0.3, 0.4, 0.55, 0.7, 0.8, and 0.9 mL) delivered to the balloon placed within the vagina of the rats. Escape responses reflect the percentage of times rats extended their head into the tube to interrupt the light beam. A significance threshold of 0.05 was applied to Bonferroni–corrected p-values.

Additional resources

The animal model study and procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) as protocol #2021000201. The electronic medical records study was approved by the University of California, San Francisco, institutional review board (as IRB number 22-37954) and considered secondary research for which consent is not required.