Prediction of Differential Pharmacologic Response in Chronic Pain Using Functional Neuroimaging Biomarkers and Support Vector Machine Algorithm – An Exploratory Study

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ABSTRACT (250/250 words)

Objective: There is increasing demand for prediction of chronic pain treatment outcomes using machine-learning models, in order to improve suboptimal pain management. In this exploratory study, we used baseline brain functional connectivity patterns from chronic pain patients suffering from Fibromyalgia (FM) to predict whether a patient would respond differentially to either milnacipran or pregabalin, two FDA-approved drugs for the treatment of FM.

Methods: FM patients participated in two separate double-blind placebo controlled crossover studies of milnacipran (N=15) and pregabalin (N=13), respectively. Functional magnetic resonance imaging during rest was collected before treatment to measure intrinsic functional brain connectivity in several brain regions involved in pain processing. A support vector machine algorithm was used to classify FM patients into responders, defined as those patients with a 20% or greater improvement in clinical pain, to either milnacipran or pregabalin.

Results: Connectivity patterns involving the posterior cingulate and dorsolateral prefrontal cortex individually classified pregabalin responders versus milnacipran responders with 77% accuracy. Performance of this classification improved when both posterior cingulate and dorsolateral prefrontal connectivity patterns were combined, resulting in a 92% classification accuracy. These results were not related to confounding factors including head motion, scanner sequence, or hardware status. Connectivity patterns failed to differentiate drug non-responders across the two studies.

Conclusion: Brain functional connectivity patterns used in a machine learning framework differentially predicted clinical response to pregabalin and milnacipran in chronic pain patients. These findings highlight the promise of machine learning in pain prognosis and treatment prediction.

Trial Registration: Clinicaltrials.gov numbers NCT01173055 and NCT00760474
Introduction

Suboptimal management of chronic pain has contributed to a pain-related health crisis, including the ongoing opioid epidemic in the United States. As a result, discovery of biological markers of pain to supplement self-report measures of clinical pain has been garnering attention, and become a priority for organizations like the National Institutes of Health (e.g., the HEAL initiative). Ultimately, such biomarkers will aid diagnosis, forecast longitudinal outcomes, and predict treatment efficacy.

One clinical pain disorder where biomarker development has become imperative is fibromyalgia (FM), a chronic condition characterized by widespread pain, fatigue, hypersensitivity to sensory stimuli (1), and increased prevalence of multiple negative health outcomes. Research in the past two decades has shown augmented pain and sensory processing in the central nervous system is a primary mechanism underlying pain in FM patients (2, 3). Multiple brain loci have been identified as being related to FM pain, including subregions of the salience, default mode, and somatosensory networks (3, 4). Some regions of the default mode network (DMN) have been shown to have
increased connectivity to the salience and somatosensory networks in FM. This type of connectivity might be a marker for chronic pain intensity in this population (3, 4).

Functional magnetic resonance imaging (fMRI) studies have demonstrated that aberrant pain processing in FM can be modulated with FDA-approved pharmacological compounds such as pregabalin, milnacipran, and duloxetine. However, only 30% of FM patients report clinically significant pain improvements with either of these drugs (5-7). Patients who fail to receive immediate analgesic effects may receive other treatments in a ‘trial and error’ approach that is both inefficient and costly. The identification of tools that can predict effectiveness of specific pharmacological agents used to treat pain at the individual patient level would be of significant clinical benefit and an important step towards personalized analgesia.

With the advent of sophisticated multivariate data analytic techniques such as machine-learning, prediction of analgesic response from neuroimaging data has become a promising avenue for biomarker development (8). Briefly, machine-learning techniques “learn” the underlying data patterns (e.g., neuroimaging voxels) to form a model using labels (e.g., pregabalin responder versus milnacipran responder), which can then be applied to unseen/new data to make predictions. Such models have been rarely used in clinical pain populations to predict treatment efficacy. To the best of our knowledge, this study is the first to assess fMRI-derived biomarkers as predictors of differential analgesic response in chronic pain.

We built machine-learning models, using Support Vector Machine (SVM), from fMRI data obtained from two, separate, double-blind placebo-controlled crossover trials in FM patients, one with pregabalin(9) another with milnacipran(10). These two medications are thought to work differently on pain processing with pregabalin reducing pain promoting neural activity(9) and milnacipran increasing pain inhibitory pathways(10). We reasoned that given the central mechanisms of action of the two drugs, pre-treatment brain connectivity (i.e. communication between brain structures) might be able to differentially predict drug responsiveness.

Materials and Methods

Subjects
Fifty females with FM, previously enrolled into two independent double-blind placebo-controlled crossover studies investigating the effects of pregabalin versus placebo and milnacipran versus placebo (9, 10), were eligible for this secondary analysis (CONSORT diagrams are included). Twenty-seven FM patients were enrolled into the original pregabalin study. Fourteen patients were excluded (nine were excluded in the original study) (9). Five additional patients were excluded: four for head motion using more stringent translational or rotational thresholds after assessment of brain images (see below for additional details), and one for incomplete clinical data. This resulted in thirteen patients taking pregabalin included in the present analysis. Twenty-three female FM patients were enrolled into the original milnacipran study (eight were excluded as previously reported) (10). Brain images for the remaining fifteen patients passed quality inspection and were included in the present analysis.

All study participants gave written informed consent. Study protocols and informed consent documents were approved by the University of Michigan Institutional Review Board (Ann Arbor, Michigan), and the sponsor of the respective studies: Pfizer (Groton, CT) for pregabalin and Forest Laboratories (New York, NY) for milnacipran. All clinical symptom data from both trials were previously verified for accuracy and the database was locked before analysis. Neuroimaging data were stored, validated, analyzed, and assessed for quality at the University of Michigan independent of Pfizer and Forest Laboratories personnel. Patient demographics, medications, inclusion/exclusion criteria, and treatment effects have been previously reported (9, 10). Patient demographics and medications are listed in Table 1 while brief descriptions of inclusion and exclusion criteria are included in Supplemental Methods.

Clinical Pain and Mood Measures

For participants enrolled in the pregabalin study, clinical pain was assessed using a 10-cm visual analog scale (VAS) bounded by the anchors “no pain” and “worst pain imaginable”. Subjects from the milnacipran study reported their clinical pain with an itemized question from Brief Pain Inventory (BPI) that ranged from 0-10, where 0 was anchored with the words “no pain” and 10 was anchored with the words “pain as bad as you can imagine” (11). Depression and anxiety were assessed using the Hospital Anxiety and Depression Scale (HADS), a 14-item measure with each item
rated on a 4-point severity scale (12). The HADS produces two scales, one for anxiety and one for depression. The BPI, VAS, and HADS were administered prior to the baseline neuroimaging session. Differences in clinical pain and mood were measured using paired sample t-tests (pre-drug versus post-drug). Drug responders in both studies were designated as having a reduction in clinical pain of ≥20% from pre-drug to post-drug, as this criterion provided a sufficient number of subjects who did respond to either drug for meaningful SVM classification (see below). Anxiety/depression responders in both studies were designated as having a decrease in anxiety or depression and a non-responder was defined as having no change or increase in anxiety or depression.

Resting State Functional Connectivity Magnetic Resonance Imaging (fcMRI) as Prediction of Drug Response

Data Acquisition

Functional connectivity magnetic resonance imaging sessions, including a six-minute resting state scan and a high-resolution T1 structural scan, were acquired for all participants at baseline. All scans were performed on a 3.0T GE Signa Scanner (LX VH3 release, quadrature birdcage transmit-receive radio frequency coil, neuro-optimized gradients). Resting state fMRI data for both studies were acquired using a custom T2*-weighted spiral-in sequence (repetition time [TR]=2000ms, echo time [TE]=30ms, flip angle=90°, matrix size=64x64 with 43 slices, and 3.13x3.13x3mm voxels, 5 discarded dummy acquisitions). During each resting state scan (180 volumes), participants were asked to remain awake with eyes open. To reduce head motion, participants’ heads were secured in the head coil using foam padding around the sides of the head and a strap across the forehead. A fixation cross was displayed on a presentation screen. Participants were asked to lie still and fixate on the cross throughout the scan. It has been shown that cognitive tasks such as starting at a cross do not typically disrupt resting state networks(13). Physiologic data were collected simultaneously with fMRI data because cardiorespiratory fluctuations are known to influence fMRI intrinsic connectivity within several brain networks. Cardiac data were collected for each participant using an infrared pulse oximeter (GE) attached to the right middle finger. Respiratory volume data were acquired using a GE magnetic resonance-compatible chest plethysmograph that was secured around the abdomen. Further,
previously mentioned T1 high-resolution images were acquired using a spoiled gradient echo inversion recover sequence (pregabalin: repetition time [TR]=10.5ms, echo time [TE]=3.4ms, flip angle=25°, matrix size=256x256 with 106 slices, and 0.94x0.94x1.5mm voxels; milnacipran: repetition time [TR]=1400 ms, echo time [TE]=1.8 ms, flip angle=15°, matrix size=256x256 with 124 slices, and 1.02x1.02 x1.2 mm voxels). Inspection of individual T1 MR images revealed no gross morphologic abnormalities for any participant.

Preprocessing

Data were preprocessed and analyzed using FSL (www.fmrib.ox.ac.uk/fsl) and Statistical Parametric Mapping (SPM, version 8, Functional Imaging Laboratories, London, UK) as well as the functional connectivity toolbox Conn (Cognitive and Affective Neuroscience Library, Massachusetts Institute of Technology, Cambridge, USA)(14) and the GIFT toolbar(15). Following collection of functional data, cardiorespiratory artifacts were corrected for using RETROICOR(16). Subsequent preprocessing steps were conducted within SPM and included motion correction (realignment to the first image of the time series), registration of all images to the mean motion corrected functional image, normalization to the standard SPM Montreal Neurological Institute template (generating 2 x 2 x 2mm resolution images), and spatial smoothing (convolution with an 8mm FWHM Gaussian kernel). Head motion from each participant was assessed by evaluating three translations and three rotations. Translational thresholds were set to ±2mm. Rotation thresholds were limited to ±1°. A subject was to be excluded from an analysis if head motion exceeded either of the thresholds in one of the six dimensions. Seed-to-whole brain functional connectivity maps were generated using the Conn toolbox(14). Within the Conn toolbox, seed regions’ time series were extracted; white matter, cerebrospinal fluid, and realignment parameters were entered into the analysis as covariates of no interest. A band-pass filter (frequency window: 0.01-0.1 Hz) was applied, thus removing linear drift artifacts and high frequency noise. First level analyses were performed correlating seed region time series signal with averaged time series voxel signal throughout the whole brain, thereby creating bivariate Fisher z-transformation correlation seed region to voxel connectivity maps (one map per seed per individual). Machine-learning analyses were implemented using a linear SVM, performed using the libsvm toolbox version 3.18 (17) in MATLAB 7.5b.
Our prior studies (9, 10) identified regions with functional connectivity patterns that were related to drug response to pregabalin and milnacipran. We therefore chose these as seed regions to test responders to the two drugs in a machine-learning framework for prediction. These seed regions encompass various known ascending and descending pain circuits in the brain. Seed-to-whole brain functional connectivity maps were generated for the following regions (Supplementary Table 1): left posterior cingulate cortex (PCC), and left inferior parietal lobule (IPL) (based on pregabalin study results)(9); and bilateral periaqueductal gray (PAG), subgenual anterior cingulate cortex (sgACC), perigenual anterior cingulate cortex (pgACC), dorsal anterior cingulate cortex (dACC), and bilateral dorsolateral prefrontal cortex (dlPFC) (based on milnacipran study results)(10).

Support Vector Machine Classification

Machine-learning analyses were implemented using a linear SVM, performed using the libsvm toolbox version 3.18 (https://www.csie.ntu.edu.tw/~cjlin/libsvm/) in MATLAB 7.5b. Briefly, linear SVM tries to separate two separate classes (i.e. pregabalin responder versus milnacipran responder) of features (i.e. data from brain voxels) by creating a hyperplane that separates the two classes in the most optimal fashion. SVM was implemented on brain connectivity maps of pregabalin responders versus milnacipran responders. SVM classification was performed using a linear kernel with parameter C=1 (no improvement was found using a C parameter line search), and while it is true that a nonlinear kernel may capture higher order features, we found no advantage using a radial basis function with this dataset. Leave-one-subject-out cross-validation was used to calculate classification accuracies and predicted values. Accuracies exceeding 75% for identifying a drug responder were deemed clinically significant. SVM model weights were averaged across all leave-one-subject-out iterations to investigate the spatial distribution of the classification weights. Significance levels for the model weights were generated by permuting the treatment labels 100 times for each leave-one-subject-out instance, resulting in 1300 model weight instances for each voxel location for the pregabalin responder versus milnacipran responder analysis, allowing significance to be calculated by the number of times a model weight occurred in the histogram. Significant values (p<0.05) were overlaid on reference anatomy, and the connectivity maps of the most significant areas were plotted to examine their relationship to the multivariate pattern. A chance-level classification outcome was taken to reaffirm that the given predictive model was specific and minimally affected by confounds.
To determine if pre-drug VAS ratings can predict responders to pregabalin from responders to milnacipran with high accuracy, we performed a logistic regression analysis. Then, to investigate if there was an additive effect on classification accuracy, we included pre-drug clinical pain ratings as a vector to the connectivity maps of each patient and performed SVM classification.

Assessment of Confounds and Investigation of Separation Accuracy in Support Vector Machine Classification

Subsequent steps were taken to confirm that a classifier did not possess any confounds: (a) for specificity of pregabalin versus milnacipran classifier to responders, the model was also tested in the non-responders, (b) since milnacipran is an antidepressant, we also investigated whether the classifier is specific to predicting changes in pain as opposed to changes in anxiety or depression using the Hospital Anxiety and Depression Scale questionnaire (12), the model was tested first, in pregabalin responder versus milnacipran responder for depression, and second, between responder and non-responder labels for anxiety and depression (where a responder was defined as having a decrease in anxiety or depression and a non-responder was defined as having no change or increase in anxiety or depression), (c) to investigate whether subject motion was influencing the classification, high motion versus low-motion, and finally (d) to confirm that the classifier was not predicting differences between sequence and hardware status from the pregabalin and milnacipran studies, an SVM analysis was performed comparing baseline placebo scans for all seeds between the two studies. This SVM analysis was performed exactly as was described above for the responder versus responder analysis above except the input was placebo data for all subjects from both studies (pregabalin N=13; milnacipran N=15).

Results

Subject Demographics, Clinical Pain, and Psychological Measures

There was no significant difference in age between patients in the two studies (mean ± SD yrs; pregabalin 35.7 ± 11.4; milnacipran 40.7 ± 10.2; p=0.228). Pregabalin responders (N=6) and milnacipran responders (N=7) reported less pain following drug compared to non-responders. Full results of clinical pain and effects of responders, non-responders and all patients are found in Table 2.
Results of anxiety and depression for these aforementioned groups (responders/non-responders determined by clinical pain improvement) are included in Table 2. We focused our analyses on the ability of pre-treatment fcMRI to predict differential analgesic responsiveness.

**Differential Classification of Response to Pregabalin versus Milnacipran**

We were unable to predict differential response to pregabalin versus milnacipran using pre-drug clinical pain ratings (62% accuracy; Beta = -0.065, p = 0.782). Whole-brain connectivity patterns to the left PCC seed classified pregabalin responders from milnacipran responders with 77% accuracy (Figure 1A). Significant average model weights contributing to successful classification included greater connectivity for pregabalin responders versus milnacipran responders to regions such as the left IPL, left precuneus, left and right PCC, right perigenual anterior cingulate cortex, and right primary motor/somatosensory cortex. In addition, significant average model weights where connectivity to the left PCC was found to be greater for milnacipran responders as compared to pregabalin responders included the left primary visual cortex, bilateral superior medial frontal gyrus, bilateral superior parietal lobule, and the right superior temporal gyrus (Figure 1A, Table 3).

Whole-brain connectivity patterns of the right dlPFC classified pregabalin responders from milnacipran responders with 77% accuracy (Figure 1B). Significant average model weights contributing to successful classification included greater connectivity for pregabalin responders versus milnacipran responders to regions including the left precuneus, bilateral superior and inferior parietal lobules, left middle and superior frontal gyri, right superior frontal gyrus and the left posterior insula cortex (Table 3). Additionally, significant average model weights where connectivity of the dlPFC was found to be greater in the milnacipran responders as compared to the pregabalin responders included the right anterior cerebellum, right superior frontal gyrus, right middle temporal gyrus, and the right superior temporal gyrus. Many of these connected regions are found to be involved in the frontoparietal, dorsal attention, and sensorimotor networks. The remaining seed regions included in this study did not yield high predictive accuracy. When adding pre-treatment clinical pain ratings as an additional feature, this combination model did not improve the predictive power beyond using the whole-brain connectivity maps alone (left PCC = 77% accuracy, right dlPFC = 77% accuracy).
In order to enhance classification performance of the aforementioned models, a combinatorial SVM model was produced using connectivity maps from both the left PCC and the right dlPFC. This combinatorial classification model was able to synergistically classify responders to pregabalin from responders to milnacipran with 92% accuracy (Figure 1C). Significant average model weights contributing to this result included the combination of those brain regions previously described individually – regions within the DMN such as the precuneus, PCC, and IPL, the mid cingulate cortex, the posterior insula cortex, the perigenual anterior cingulate cortex, and multiple regions within the cerebellum (Figure 1). Complete results are found in Table 3. As with our PCC and dlPFC models alone, the addition of the pre-treatment clinical pain ratings as an additional feature did not improve the predictive power of the combined SVM model included left PCC + right dlPFC yielding 92% accuracy.

Assessment of Confounds and Investigation of Separation Accuracy in Support Vector Machine Classification

To confirm these significant average model weights of connectivity were specific to responders and not non-responders for these compounds, the average model weights from these analyses were then applied to the non-responders from both pregabalin (N=7) and milnacipran (N=8). When performed, there were below chance classification accuracies of 47% for the PCC connectivity maps and 40% for the dlPFC, with the remaining seeds resulting in classification accuracies found to be less than the relevant clinical threshold set for this study (range of 13% to 73%).

We also confirmed that classification weights were specific to pain and not related to changes in anxiety and depression following treatment. In this pregabalin responder versus milnacipran responder analysis, the PCC and dlPFC maps did not yield significant classification for anxiety (left PCC: 15%, right dlPFC: 54%) or depression (left PCC: 54%, right dlPFC: 54%). In an exploratory analysis, responder and non-responder labels were created for anxiety and depression scores for both pregabalin and milnacipran groups. The only significant classification in milnacipran was for pgACC connectivity that classified responders versus non-responders in terms of depression scores, with 73% accuracy (Supplementary Figure 1 and Supplementary Table 2).
Furthermore, we confirmed that head motion during acquisition of fMRI images did not confound classification performance. For each subject, a composite motion value was created from the average of all timepoints along the 6 dimensions (3 rotation and 3 translation). This was used to split subjects into high or low motion groups. The goal was to see if the prediction model obtained from pregabalin responders vs. milnacipran responders was able to predict high and low motion labels. All comparisons yielded below chance classification, ranging from 8-31% accuracy, demonstrating that the drug classification models obtained from brain images were not confounded by head motion.

We wanted to be sure our classifier was not predicting sequences or MRI hardware differences between the pregabalin and milnacipran studies despite both studies using the same scan sequences and MRI scanner. When adding baseline placebo scans from both studies, we were unable to classify between fcMRI data from the milnacipran study (N=15) and the pregabalin study (N=13) for every seed included in this study with high accuracy (accuracy values ranged from 25% to 64%).

Discussion

FM is a complex condition that is difficult to treat, with pharmacologic interventions only providing significant pain relief in a minority of cases. There has been a recent surge of interest in utilizing candidate pain biomarkers in a predictive, machine-learning framework to improve the management of FM and related chronic pain conditions (18). Here, we utilized machine-learning on resting state fMRI data collected from two cohorts of FM patients who underwent longitudinal therapy with pregabalin or milnacipran. The two drugs have differential neurochemical properties and mechanisms of action in the central nervous system – pregabalin is thought to act through inhibition of calcium-dependent release of excitatory neurotransmitters, whereas milnacipran likely works through increasing norepinephrine and serotonin signaling in descending inhibitory pathways (9, 10). Therefore, we sought to understand if functional connectivity between brain regions implicated in pain processing and modulation may be a candidate biomarker that predicts differential response to pregabalin and milnacipran in patients with FM. Machine-learning models showed baseline patterns of brain connectivity distinguished responders to pregabalin and milnacipran, significantly above chance-level. To further distinguish that these markers were specific to pain only, we confirmed that
improvements in anxiety (left PCC: 15%, right dlPFC: 54%) and depression (left PCC: 54%, right dlPFC: 54%) were not identified by our classifiers. Moreover, motion, scanner and sequence parameters did not seem to contribute to our results. Finally, our approach did not classify non-responsiveness to the two drugs, suggesting that our markers predict differential analgesic response.

Our results highlight classification differences between milnacipran and pregabalin. We assessed brain connectivity patterns of the posterior cingulate, a key node of the DMN, and found that within-DMN connectivity patterns were higher in pregabalin responders than in milnacipran responders. Interestingly, our PCC seed region was placed in a dorsal subregion of the PCC which has been found to be associated with pain widespreadness in FM (19), while the resultant connected ventral subregion of the PCC has been shown to be associated with pain castrastophizing in patients with FM (20). This suggests that our classifier identified multiple DMN regions that influence chronic pain. Further, we explored connectivity of the dlPFC, an antinociceptive node that has shown modifications in connectivity with treatment in previous studies of chronic pain (21). Here we observed that greater connectivity of the dlPFC with subregions of networks including frontoparietal, dorsal attention, sensorimotor networks differentially predict pregabalin from milnacipran responders.

Recent machine learning neuroimaging studies in chronic pain have shown that combining data across different tasks (22) or modalities (23) can bolster classification and prediction accuracy. We combined whole-brain seed connectivity maps of the PCC and the dlPFC and found increased classification performance (92%) in distinguishing pregabalin versus milnacipran outcomes, which is substantially higher than individual performance of either seed in isolation (77%). These results underscore the fact that these two drugs may act on different brain regions and combining results from different networks can capture unique aspects of pain pathology and bolster classification performance.

In recent years there has been a push towards personalized or precision medicine for numerous medical conditions ranging from cancer to depression (24). Given that most of these conditions are etiologically complex, there are likely multiple pathologies across individuals who share a common diagnosis. The situation is similar for chronic pain. There may be multiple pain processing pathways wherein plasticity may promote chronic pain that outlasts peripheral nociceptive drive. Therefore, the effectiveness of an analgesic, with a specific set of molecular targets, may be more or less suited to
any individual person based on their specific pathology. While the concept of personalized or precision analgesia has been discussed for over two decades (25), it still remains largely unrealized for chronic pain.

There are several limitations to this study. The sample size used in this study is small due to the design of our two previous studies, and we were unable to include an independent replication cohort. Machine-learning studies typically need larger sample sizes to be robust. Therefore, in future studies, training the model on larger sample sizes with out-of-sample validation is needed to determine if these results are reproducible. Furthermore, to optimize our SVM analyses, we used an unorthodox cutoff to identify our pregabalin and milnacipran responder groups (20% reduction in clinical pain). Studies with larger samples may be able to provide a more traditional cutoff point of 30% pain improvement to identify drug responders. Other machine learning techniques could have been chosen, but one of our main motivations to use SVM for this study was that it is designed to deal with small sample sizes and high dimensional data(26). While we were able to achieve high accuracy with leave-one-out-cross-validation, we also attempted a k-fold cross-validation analysis where we used a 5-fold cross validation. This approach yielded the same accuracy values (77% for the dIPFC and PCC independently, and 92% for the combination of the dIPFC and PCC maps) suggesting that our findings are consistent across multiple methods. Finally, only females were enrolled this study, and other factors such as age, race, and concurrent medications were not included as part of the SVM.

While our study has limitations, we see this work as a first step towards building robust, generalizable, and predictive markers of pharmacologic response in chronic pain. Machine learning combined with fcMRI is not yet ready for clinical application. There have been studies that have taken steps to close this gap (27), but it is yet to be confirmed as a viable tool in a clinical setting. To this end, ongoing work will investigate using whole brain correlation matrices, feature selection techniques, and nonlinear kernels as additional approaches to analgesic prediction.

In summary, our results demonstrate that brain connectivity at baseline, prior to commencing therapy, may be leveraged to differentially predict responders between analgesics. The predictive ability may be due to the mechanism of action of these pharmacological agents on the endogenous pain circuits in the central nervous system. Larger, multisite, and systematic trials with multimodal
biomarkers are needed in the future to validate these findings and utilize them in a precision medicine framework.

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions and exceptions, Pfizer may also provide access to the related individual anonymized participant data.

See https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information.

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Figure Legends

Figure 1. Resting state connectivity predicts responders to pregabalin and responders to milnacipran with high accuracy

Baseline resting state functional connectivity between seed (PCC and dlFPC)-to-whole brain, classifies responders to pregabalin from responders to milnacipran. Warm colors (red-to-yellow) designate positive weights where milnacipran responders have greater connectivity compared to pregabalin responders and regions in cool colors (darker blue-to-light blue) designate negative weights where pregabalin responders have more connectivity compared to milnacipran responders. The graphs display predictions of pregabalin responders (blue circles) and milnacipran responders (red circles).

Panel (A) displays baseline resting state functional connectivity between the left posterior cingulate cortex seed and regions including the precuneus, inferior parietal lobule, posterior cingulate cortex, and left insula cortex classifies pregabalin responders versus milnacipran responders with 77% accuracy.

Panel (B) displays baseline resting state functional connectivity between the right lateral dorsolateral prefrontal cortex seed and regions including superior parietal lobule, precuneus, primary somatosensory cortex, and left insular cortex classifies pregabalin responders versus milnacipran responders with 77% accuracy.

Panel (C) displays a combination model where baseline resting state functional connectivity between the left posterior cingulate cortex, the right dorsolateral prefrontal cortex seeds and the superior parietal lobule, precuneus, perigenual anterior cingulate cortex, mid cingulate cortex, and posterior cingulate cortex classifies pregabalin responders versus milnacipran responders with 92% accuracy.
| Patient | Age | Race | BMI | Medications and Supplements |
|---------|-----|------|-----|-----------------------------|
| 1       | 44  | White| 25  | Augmentin/Motrin             |
| 2       | 29  | White| 21  | Albuterol/Erythromycin Eye lotion/Extra Strength Tylenol/Ibuprofen/Ortho Tri-Cyclen/Zantac/Zyrtec |
| 3       | 25  | White| 23  | Children’s Tylenol Plus Cough and Running Nose/Motrin |
| 4       | 43  | White| 25  | Triamcinolone acetonide 0.5% |
| 5       | 36  | White| 21  | Amoxicillin/Augmentin/Motrin/Synthroid/Tylenol |
| 6       | 42  | White| 27  | Sudafed/Tylenol/Zyrtec      |
| 7       | 42  | White| 26  | Advil/CVS Sinus Allergy/Effexor/Nyquil/Tylenol |
| 8       | 39  | White| 25  | Claritin/Melatonin/Nuvaring/Propionate Fluticasone/Tylenol |
| 9       | 44  | White| 30  | Amoxicillin/Nyquil/Prednisone/Proventil/Rocephin/Tylenol |
| 10      | 59  | White| 29  | Colchicine/Flexeril/Hydrochlorothiazide/Melatonin/Nabumetone/Omacor/Prilosec Trazadone |
| 11      | 19  | White| 23  | Bupropion /Concerta /Loestrin/ |
| 12      | 19  | White| 26  | Claritin/Concerta/Loestrin    |
| 13      | 39  | White| 25  | Effexor/Excedrin ES/Fluticasone Propionate Nasal Spray/Ibuprofen/Maxalt/Proventil HFA/Pseudoephedrine/Seasonale/Topomax/ Zonisamide |
| Patient | Age | Race       | BMI | Medications and Supplements                                                                 |
|---------|-----|------------|-----|---------------------------------------------------------------------------------------------|
| 1       | 54  | White      | 33  | Tramadol                                                                                     |
| 2       | 26  | White      | 36  | Pregabalin/Metformin                                                                        |
| 3       | 30  | White      | 31  | Metronidazole/ Benadryl/Motrin                                                               |
| 4       | 42  | White      | 27  | Ibuprofen/Sudafed                                                                           |
| 5       | 53  | White      | 33  | Di-nox/Anacin/Aleve/Ibuprofen/Pednisone/Mobic/Mucinex/Ventolin/Airborne                      |
| 6       | 36  | African American | 37 | Amlodipine Besylate/Lisinopril-HCTZ/Aleve                                                    |
| 7       | 40  | White      | 24  | Nuvaring/Ibuprofen/Skelaxin/Vicodin/Tylenol/Quasense                                         |
| 8       | 36  | White      | 21  | Synthroid/Tylenol/ Acetaminophen/Motrin/Ibuprofen                                            |
| 9       | 39  | White      | 21  | Nasonex                                                                                      |
| 10      | 50  | White      | 29  | Lisinopril/hctz/Amoxicillin                                                                 |
| 11      | 30  | White      | 28  | Xanax/Cataphlam/Aleve                                                                       |
| 12      | 40  | White      | 23  | Motrin                                                                                       |
| 13      | 53  | African American | 26 | Motrin/Excedrin                                                                              |
| 14      | 27  | White      | 32  | Levothyroxine/ Singulair/Albuterol                                                         |
|         |     |            |     | Sulfate/Vicodin/Cyclobenzabrine/Maxalt/Tylenol/Cortizone shots/Cephalexin                   |
Patient demographic information from the pregabalin and milnacipran studies are displayed including age, race, body mass index and medications taken.

Table 2: Clinical Pain, Anxiety, and Depression Information for All Patients and Responders/Non-Responders based on Pain Improvement

| Clinical Pain Data | Pre-treatment, Mean (SD) | Post-Treatment, Mean (SD) | P Value |
|--------------------|--------------------------|---------------------------|---------|
| Pregabalin Study – Clinical Pain, VAS 0-10 cm | | | |
| Pregabalin responders | 5.4 (2.5) | 1.6 (2.3) | 0.0003 |
|                          | Pregabalin non-responders | Pregabalin all patients | P Value |
|--------------------------|---------------------------|-------------------------|---------|
|                          | 1.3 (1.0)                 | 1.7 (2.3)               | 0.60    |
|                          | 3.2 (2.7)                 | 1.7 (1.9)               | 0.06    |

**Milnacipran Study – Clinical Pain, VAS 0-10 cm**

|                          | Milnacipran responders | Milnacipran non-responders | Milnacipran all patients | P Value |
|--------------------------|------------------------|---------------------------|-------------------------|---------|
|                          | 5.6 (1.5)              | 4.8 (2.4)                 | 5.1 (2.0)               | 0.00009 |
|                          | 2.1 (2.0)              | 6.0 (2.6)                 | 4.2 (3.0)               | 0.23    |

### Pre-treatment Clinical Pain, VAS 0-10 cm

|                          | Pregabalin, Mean (SD) | Milnacipran, Mean (SD) | P Value |
|--------------------------|------------------------|-------------------------|---------|
| Responders               | 5.4 (2.5)              | 5.6 (1.5)               | 0.85    |

### Anxiety and Depression Data

|                          | Pre-treatment, Mean (SD) | Post-Treatment, Mean (SD) | P Value |
|--------------------------|--------------------------|---------------------------|---------|
| **Pregabalin Study – Anxiety, HADS Anxiety Score** |                          |                           |         |
| Pregabalin responders    | 9.5 (2.3)                | 7.8 (4.9)                 | 0.31    |
| Pregabalin non-responders| 2.9 (2.9)                | 2.6 (3.2)                 | 0.17    |
| Pregabalin all patients  | 5.9 (4.3)                | 5.0 (4.7)                 | 0.40    |
|                  | Pregabalin Study – Depression, HADS Depression Score |                  |                  |
|------------------|--------------------------------------------------|------------------|------------------|
|                  | Pregabalin responders | 6.5 (3.3) | 4.0 (3.8) | 0.22 |
|                  | Pregabalin non-responders | 1.7 (2.2) | 2.3 (2.4) | 0.46 |
|                  | Pregabalin all patients | 3.9 (3.6) | 3.1 (3.1) | 0.40 |
|                  | Milnacipran Study – Anxiety, HADS Anxiety Score |                  |                  |
|                  | Milnacipran responders | 7.0 (3.5) | 3.9 (3.1) | 0.01 |
|                  | Milnacipran non-responders | 6.1 (2.5) | 5.6 (3.3) | 0.53 |
|                  | Milnacipran all patients | 6.5 (2.9) | 4.8 (3.3) | 0.02 |
|                  | Milnacipran Study – Depression, HADS Depression Score |                  |                  |
|                  | Milnacipran responders | 4.9 (3.0) | 3.1 (2.9) | 0.03 |
|                  | Milnacipran non-responders | 4.1 (2.5) | 4.5 (2.1) | 0.53 |
|                  | Milnacipran all patients | 4.5 (2.7) | 3.9 (2.5) | 0.24 |
|                  | Pregabalin, Mean (SD) | Milnacipran, Mean (SD) | P Value |
| Pre-treatment HADS Anxiety Score |                  |                  |                  |
| Responders | 9.5 (2.3) | 7.0 (3.5) | 0.16 |
| Pre-treatment HADS Depression Score |                  |                  |                  |
Responders were defined as having a ≥ 20% reduction in clinical pain (VAS) following treatment with drug. The pregabalin sample included 13 patients (46% were responders: N = 6; 54% were non-responders: N = 7). The milnacipran sample included 15 patients (47% were responders: N = 7; 53% were non-responders: N = 8).

| Seed Region                       | Accuracy | Significant Weights                                  | Size   | Coordinates |
|-----------------------------------|----------|------------------------------------------------------|--------|-------------|
| Left posterior cingulate cortex   | 77%      | Left inferior parietal lobule - BA 40 (-)            | 19488  | -52 -50 44 |
|                                   |          | Left precuneus (-)                                   |        | -6 -68 38  |
|                                   |          | Left ventral posterior cingulate cortex (-)          |        | -6 -46 14  |
|                                   |          | Right medial frontal gyrus (-)                       | 18984  | 6 60 0     |
|                                   |          | Right perigenual anterior cingulate cortex (-)       |        | 8 44 -2    |
|                                   |          | Right inferior parietal lobule (-)                   | 4800   | 46 -50 44  |

Table 3: Significant Multivariate Prediction of Baseline Resting State Connectivity Between Pregabalin Responders and Milnacipran Responders
| Region                                           | MNI Coordinates |
|-------------------------------------------------|-----------------|
| Right medial frontal gyrus/superior frontal gyrus (-) | 3296 16 32 42   |
| Left primary visual cortex (+)                  |                 |
| Right primary motor/primary somatosensory cortex (-) | 2744 38 -30 62  |
| Left posterior cerebellum - Crus 1, Crus2 (-)    | 2728 -40 -66 -42 |
| Left posterior insula cortex/superior temporal gyrus (-) | 1512 -40 -26 14 |
| Right posterior insula cortex (-)                | 744 50 -10 8    |
| Left superior frontal gyrus (-)                  | 736 -20 32 46   |
| Right/left superior medial frontal gyrus (+)     | 680 2 60 30     |
| Right superior parietal lobule (+)               | 640 26 -60 60   |
| Right superior parietal lobule (+)               | 448 56 8 -8     |
| Left superior parietal lobule (+)                | 360 -32 -66 60  |
| Right putamen (-)                                | 328 26 16 -2    |
| Right midbrain/pons (-)                         | 328 4 -24 -20   |
| Right lateral dorsolateral prefrontal cortex (dlPFC) | 77%             |
| Right superior parietal lobule (-)               | 36656 14 -80 54 |
| Right inferior parietal lobule (-)               | 46 -46 54       |

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| Region                                      | X  | Y   | Z   |
|--------------------------------------------|----|-----|-----|
| Left precuneus (-)                         | -4 | -66 | 44  |
| Left superior parietal lobule (-)          | -16| -70 | 56  |
| Left inferior parietal lobule (-)          | -40| -62 | 54  |
| Left primary somatosensory cortex (-)      | -50| -18 | 58  |
| Left middle frontal gyrus (-)              |  8|  18| -18 |
| Left inferior frontal gyrus (-)            | -32|  30 | -12 |
| Right superior frontal gyrus (-)           |  34|  44 | -16 |
| Right pons (-)                             | 1800|  20 | -14 |  34 |
| Right parahippocampal gyrus (-)            |  22|  -4 | -28 |
| Left pons (+)                              | 1592|  16 | -50 | -2  |
| Right anterior cerebellum (+)              |  10| -48 | -28 |
| Left precentral gyrus (-)                  | 1000| -50 |  2  |
| Right superior frontal gyrus (+)           |  72|  16 |  26 |
| Left inferior occipital gyrus (+)          |  54| -44 |  64 |
| Right middle temporal gyrus (+)            |  46|  52 | -6  |
| Right superior temporal gyrus (+)          |  45|  42 | -22 |
| Right posterior cerebellum (+)             |  44| -46 | -22 |
| Left posterior insula cortex (-)           |  36| -34 | -28 |

Left PCC and R dlPFC 92% Right superior parietal lobule (-) 36928 34 -50 62
| Brain Region                                | X    | Y    | Z    |
|--------------------------------------------|------|------|------|
| Right primary somatosensory cortex (-)     | 58   | -18  | 48   |
| Right inferior parietal lobule – BA 40 (-) | 44   | -40  | 46   |
| Left precuneus (-)                         | -8   | -64  | 44   |
| Right precuneus (-)                        | 6    | -62  | 42   |
| Left inferior parietal lobule – BA 40 (-)  | -40  | -50  | 46   |
| Right perigenual anterior cingulate cortex (-) | 19408 | 4    | 36   | -4   |
| Right medial frontal gyrus (-)             | 4    | 58   | -6   |
| Left inferior parietal lobule – BA 40 (-)  | 18800| -48  | -50  | 44   |
| Left precuneus (-)                         | -10  | -62  | 42   |
| Left ventral posterior cingulate cortex (-) | -2   | -46  | 20   |
| Left mid cingulate cortex (-)              | -2   | -34  | 46   |
| Left inferior orbital frontal gyrus (-)     | 9672 | -26  | 30   | -10  |
| Right mid orbital frontal gyrus (-)         | 18   | 48   | -10  |
| Left precuneus (-)                         | 5072 | -6   | -42  | 70   |
| Right mid cingulate (-)                    | 6    | -28  | 44   |
| Right primary somatosensory cortex (-)     | 2160 | 50   | -28  | 62   |
| Right anterior cerebellum (+)              | 1376 | 10   | -48  | -24  |
| Right pons (+)                              | 8    | -30  | -36  |
| Right supplementary motor area (-)          | 1176 | 36   | 0    | 58   |
| Left cuneus (+)                             | 1136 | -14  | -78  | 8    |
| Brain Region                              | X   | Y   | Z   | T   |
|------------------------------------------|-----|-----|-----|-----|
| Left superior frontal gyrus (-)          | 880 | -16 | 28  | 52  |
| Right supplementary motor area (+)       | 880 | 14  | 24  | 64  |
| Left posterior cerebellum (+)            | 760 | -6  | -78 | -34 |
| Left inferior occipital gyrus (+)        | 696 | -48 | -82 | -4  |
| Left posterior cerebellum (+)            | 496 | -42 | -52 | -50 |
| Right mid temporal gyrus (+)             | 488 | 50  | -4  | -20 |
| Left superior parietal lobule (+)        | 472 | 22  | -56 | 62  |
| Left mid cingulate cortex (-)            | 464 | -10 | -24 | 54  |
| Left premotor cortex (+)                 | 448 | -34 | -8  | 54  |
| Right posterior insula cortex (-)        | 416 | 44  | -10 | 10  |
| Right posterior cerebellum (+)           | 360 | 6   | -68 | -46 |
| Right superior temporal gyrus (+)        | 352 | 60  | 10  | -8  |
| Right superior frontal gyrus (+)         | 336 | 14  | 24  | 62  |

Support vector machine accuracy values were achieved from correctly identifying pregabalin “responders” and milnacipran “responders”. Group labels were chosen based on median splits (Pregabalin: responders had a 20 point or greater reduction in pain following treatment period; Milnacipran: responders had a 20 point or greater reduction in pain following treatment period), and patients deemed as responders to each respective drug were entered into this analysis. Results are significant at a p value < 0.05 derived from permutation testing (1300 iterations) reporting clusters greater than 320 mm³. (+) denotes greater functional connectivity for milnacipran responders compared to pregabalin responders. (-) denotes greater functional connectivity for pregabalin responders compared to milnacipran responders. PG = pregabalin, MLN = milnacipran, SVM = support vector machine, PCC = posterior cingulate cortex, dlPFC = dorsolateral prefrontal cortex.
