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Next-generation precision medicine for pain

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Chronic pain remains a massive problem in society in general, and in mental health patients in particular, being strongly bi-directionally connected to mental health. Lack of widespread use of objective information has hampered treatment and prevention efforts. Pain is a spectrum of severity, from transient vague discomfort to chronic excruciating incapacitation. Blood biomarkers that track pain severity can provide a window into the biology of pain, as well as could help with assessment and treatment. A previous study by us was positive. Here we describe new studies we conducted trans-diagnostically in psychiatric patients, starting with the whole genome, to expand the identification, prioritization, validation and testing of blood gene expression biomarkers for pain. We carried out two separate studies, on two different platforms, microarrays and RNA sequencing, using for each study a multiple independent cohorts design. This ensured biological and technical reproducibility. We then focused at the end on biomarkers that were convergent and reproducible between the two studies. We found new as well as previously known biomarkers that were predictive of high pain states, and of future emergency department visits related to them, using cross-sectional and longitudinal approaches. Using a polyevidence score, the overall top decreased in expression biomarker ("pain-suppressor gene") was CD55, a gene that suppresses the complement cascade and cell damage. The top increased biomarker ("algogene") was ANXA1, a gene that is an effector of glucocorticoid-mediated responses and regulator of the inflammatory processes. The top biological pathways were related to cellular response to TNF and to neuroinflammation. The top upstream regulator was TNF. Top therapeutic matches overall were the medications lithium and ketamine, as well as the nutraceuticals omega-3 fatty acids and magnesium. Drug repurposing bioinformatic analyses also identified the potential of carvedilol, sirolimus, budesonide, berbamine, and quetiapine, as well as of medications already used to treat pain such as amyleine, sulindac, sufentanil, carbamazepine, and meclofenamic acid, that serve as de facto positive controls. Additionally, we show how personalized patient reports for doctors would look like based on blood biomarkers testing, to aid with objective assessment of severity and risk, as well with individualized matching to medications and nutraceuticals. Given the fact that pain disorders are highly prevalent, can severely affect quality of life, and even lifespan, there is an urgent need for insights and tools such as the ones we have developed to be applied to and improve clinical diagnosis, treatment, and prevention options.

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INTRODUCTION

"The cure for pain is in the pain."

-Rumi

Pain disorders are common and oftentimes disabling conditions. Every year, an estimated 20% (aprox. 50 million) of U.S. adults have chronic pain and 8% of U.S. adults (aprox. 20 million) have high-impact chronic pain. Higher prevalences of both chronic pain and high-impact chronic pain were reported among women, older adults, previously but not currently employed adults, adults living in poverty, adults with public health insurance, and rural residents [1]. Psychiatric patients in particular are at high risk and have high co-morbidity for pain conditions [2]. The lack of use of objective tests in clinical practice, particularly when an obvious lesion is not present, and the lack of efficacy or addictive potential of certain medications, create barriers to proper care and improvement of patients.

Our previous studies had pioneered the identification of blood gene expression biomarkers for pain [3], and other groups have validated blood-based approaches as well [4]. Other approaches involve imaging [5], and genetic biomarkers [6]. Some understanding of the neurobiology is emerging [7]. Our gene expression studies are complementary to other genetic studies in the field, and in fact we integrate these different lines of work into our approach, as convergent evidence and prioritization second step. We wanted to expand upon our previous studies, as a way of deriving future scientific and practical insights, that would move these precision medicine

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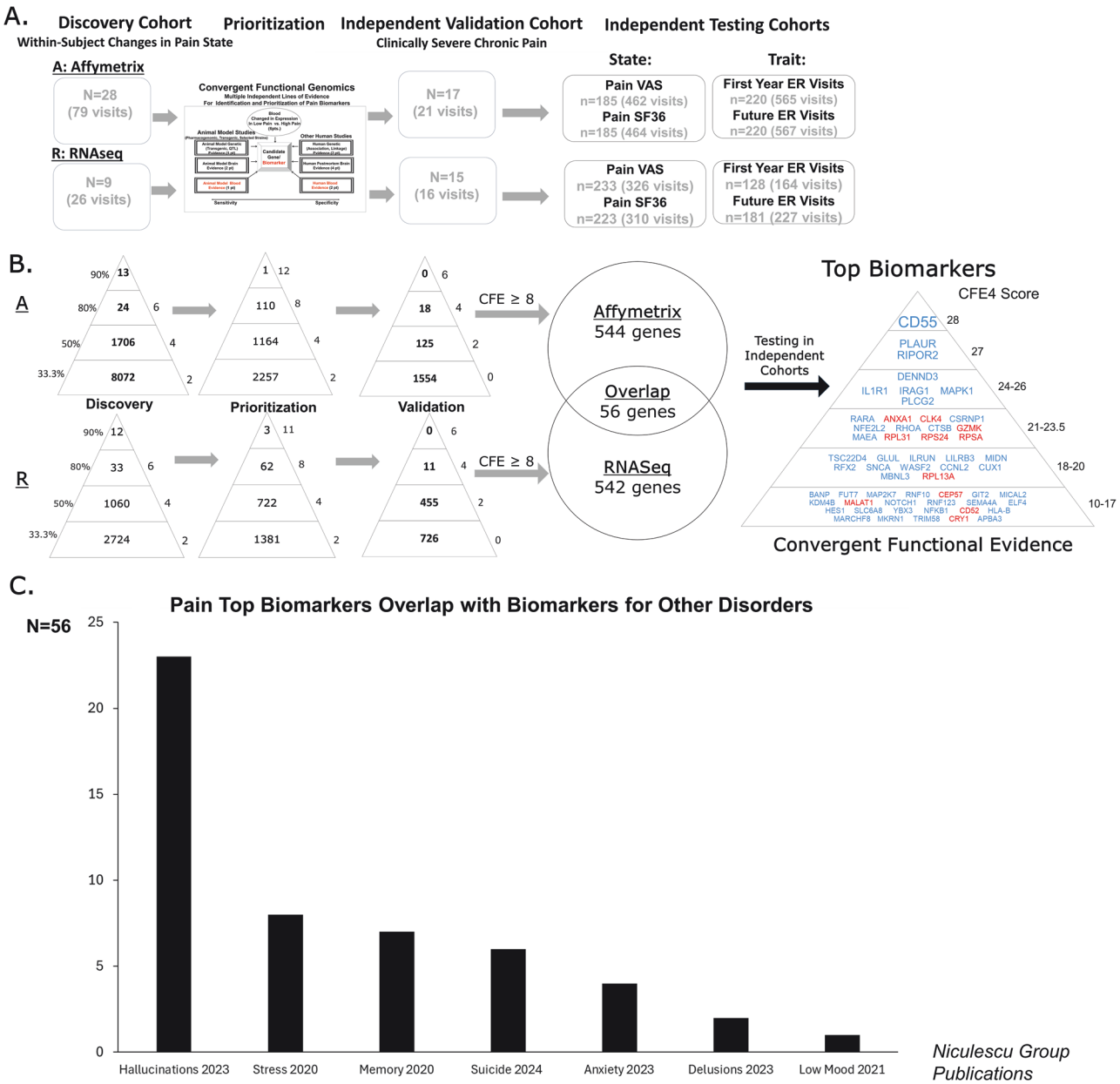


Fig. 1 Overview of the two studies and their convergence. A. Discovery, Prioritization, Validation and Testing of Biomarkers for Pain. **B**. Number of biomarkers after each step. **C**. Pain Top Biomarkers Overlap with Biomarkers for Other Disorders from Our Previous Studies.

approaches towards widespread utilization in clinical practice. We focused on convergence and reproducibility, across two different studies using two different platforms, microarrays and RNAseq, and multiple independent non-overlapping cohorts in each. For each platform, the process had 4 steps- discovery, prioritization, validation, and testing, and then we focused on convergence of findings from the two platforms. Compared to our previous work, we used larger cohorts of psychiatric patients, larger literature-derived databases for our prioritization step, and longer duration follow-up on subjects. We also used a newer technology platform, RNAseq. With this comprehensive series of studies, we derived a deeper biological, clinical, and therapeutic understanding of pain disorders.

On the practical side, diagnostically we show examples of how a report to clinicians would look like, and therapeutically we prioritize existing medications as well as identify new repurposed medications for pain. We propose that this precision medicine approach could and should be used in clinical practice, to stem and reverse the prevalence of pain disorders.

MATERIALS AND METHODS

Ethics approval and consent to participate

All subjects understood and signed informed consent forms outlining the research goals, procedure, caveats, and safeguards, per Indiana University IRB approved protocol (IU#1908417892). All research was performed n accordance with the relevant guidelines and regulations.

Cohorts

For each of the two studies, we utilized three different, non-overlapping cohorts: discovery, validation (subjects with high state pain scores (VAS Pain) and chronic pain scores (SF-36 -Q21 + Q22), and testing (an independent cohort for predicting pain and future ER visits for pain) (Fig. 1A).

Consistent with our previous studies [8–15], the psychiatric subjects were part of a longitudinal cohort collected over 20 years by us

(2004–2024), the Indy 500 + cohort. Subjects were recruited from the patient population at the Indianapolis VA Medical Center and Indiana University School of Medicine.

Subjects underwent diagnostic assessments via a comprehensive, structured clinical interview—Diagnostic Interview for Genetic Studies—at a baseline visit, followed by up to ten testing visits, 3–6 months apart or whenever a new hospitalization occurred. At each testing visit, subjects received a series of rating scales, including a VAS for Pain and the SF-36, which includes two chronic pain rating items (Q21 and Q22, related to the previous 4 weeks (Figure S1), and their blood was drawn. We collected whole blood (5 ml) in two RNA-stabilizing PAXgene tubes, labeled with an anonymized ID number, and stored at −80 °C in a locked freezer until further processing. Whole-blood RNA was extracted for microarray and RNA sequencing gene expression studies from the PAXgene tubes, as detailed below.

We conducted two separate studies, using microarrays and RNAseq, with different independent cohorts, and then looked at convergence and reproducibility of findings across the two studies.

For the microarray study, the within-subject discovery cohort consisted of 28 subjects (19 males, 9 females), with 79 testing visits. Each subject had various psychiatric disorders and multiple testing visits with at least one diametric change in VAS pain scores from low pain (VAS pain ≤ 2) to high pain (VAS pain ≥ 6), or vice versa between visits. There were 3 subjects with 5 visits, 1 subject with 4 visits, 12 subjects with 3 visits, and 12 subjects with 2 visits. For validation, there were 17 subjects with clinically severe chronic pain (9 males, 8 females) with 21 testing visits. For the independent testing cohort, there were 185 subjects (154 males, 31 females) with 464 testing visits. The cohort for predicting future ER visits with pain as a complaint consisted of 220 subjects (196 males, 24 females) with 567 testing visits, on which we had longitudinal follow-up with electronic medical records (Fig. 1).

For the RNAseq study, the within-subject discovery cohort consisted of 9 subjects (6 males, 3 females), with 26 testing visits. Each subject had various psychiatric disorders and multiple testing visits with at least one diametric change in VAS pain scores from low pain (VAS pain ≤ 2) to high pain (VAS pain ≥ 6), or vice versa between visits. There was 1 subject with 8 visits, 2 subjects with 3 visits, and 6 subjects with 2 visits. For validation, there were 15 subjects with clinically severe chronic pain (13 males, 2 females). For the independent testing cohort, there were 233 subjects (191 males, 42 females) with 326 testing visits. The cohort for predicting future ER visits with pain as a complaint consisted of 181 subjects (147 males, 34 females) with 227 visits, on which we had longitudinal follow-up with electronic medical records (Fig. 1).

Medications. Subjects within the discovery cohort had various psychiatric diagnoses and medical co-morbidities. Their medications were listed in their electronic medical records and documented at each testing visit. Subjects were on a wide variety of medications, psychiatric and non-psychiatric, however, there was no pattern of a particular class of medication. Additionally, subjects may be non-compliant with their treatment or have changes in medications or drugs of abuse not listed in their medical records. Our goal is to identify biomarkers that track pain regardless of whether the cause is due to internal biology or prompted by exogenous substances/medication. It is likely that some of the biomarkers shown in this paper are targets of medications. Furthermore, the prioritization step which occurs post discovery is based on field-wide literature convergence including genetic and animal model data which are unrelated to the effects of medication. Our design allows for discovery, validation, and replication via testing in independent cohorts of the biomarkers to occur despite differences in gender, diagnoses, medications, and other variables in the subjects.

Blood gene expression experiments

RNA extraction. Whole blood (2.5 ml) was collected via routine venipuncture and stored in PaxGene tubes containing proprietary reagents for RNA stabilization. Total RNA was then extracted and processed as previously described [8–11].

Microarrays. Microarray work was completed on a subset of subjects ($n = 794$) using previously described methodology [8–11].

All genomic data was normalized (RMA for technical variability and z-scoring for biological variability by gender) before being combined and analyzed.

RNA sequencing. Next-generation RNA sequencing studies were completed on the remaining more recent subject samples ($n = 392$). In all RNASeq samples, transcripts were required to have a minimum TPM count of 0.1. Transcripts that did not meet that criteria were discarded.

Biomarker analyses

The following analyses were performed separately for the microarray and RNASeq studies, and independent candidate biomarkers were identified. We then focused on top biomarkers that were reproducibly identified on both platforms (convergence). Our approach has built in reproducibility at every step: across studies, across platforms, across multiple independent and non-overlapping cohorts, and cross-validated with other studies in the field. Our approach is also pre-designed and pre-determined, has been used for other studies we published. We do not adjust thresholds and housekeeping genes until we get a better result. That would lead to overfitting and diminish reproducibility and generalizability.

Step 0: Housekeeping gene identification. To normalize and account for technical variance, a housekeeping gene was selected to use for normalization. We compiled a list of the most used candidate housekeeping genes in the literature and all their corresponding probesets and transcripts. Not all classic housekeeping genes are "housekeeping", i.e. invariant or biologically not involved in the disorder, depending on the phenotype. An example of that is GAPDH [16], so an empirical approach for each disease is best. A mini-Discovery was first run on the corresponding housekeeping probesets and transcripts. LDHA (200650_s_at, ENST00000227157) was the gene with the lowest scoring probeset and transcript (indicating the most invariance with specific regards to pain). Microarray and RNASeq data were then divided by their respective LDHA probeset/transcript levels.

Step 1: Discovery. For the Affymetrix cohort, a DE analysis was run at the probeset level using housekeeping normalized data, generating a raw score for each biomarker. Points were given when the probeset expression accurately corresponded to changes in pain (low to high, or high to low). The DE analysis detects how pain can be tracked by gradual changes in gene expression.

For the RNASeq cohort, the DE analysis was done at the transcript level using housekeeping normalized data.

Biomarkers decreased in expression had negative scores, and those that increased had positive scores. A value percentile (separate for increased and decreased biomarkers) was assigned to each probeset or transcript based on its final score. The percentiles were assigned by total points: ≥80%-6pts, ≥50%-4pts, ≥33.3%-2pt, <33.3%-0pts (Fig. 1). Biomarkers with a score of at least 2 points moved on to the next step, Prioritization.

Step 2: Prioritization

Databases: In our laboratory (Laboratory of Neurophenomics, www.neurophenomics.info) we have created manually curated databases of the human gene expression/protein expression studies (postmortem brain, peripheral tissue/fluids: CSF, blood and cell cultures), human genetic studies (association, copy number variations, and linkage), and animal model gene expression and genetic studies, published to date on psychiatric disorders.

Only findings deemed significant in the primary publication by the study authors, using their experimental design and thresholds, are included in the databases. Additionally, our databases only include primary literature data instead of review papers or other secondary data integration analyses to avoid redundancy. Unbiased discovery studies are favored over candidate genes hypothesis-driven studies. Our extensive databases, which are continuously updated, are used in the CFG cross validation and prioritization platform (Fig. 1).

We performed a search to identify evidence for genes involved in pain using the following keywords: pain, neuropathy, neuropathies, trigeminal neuralgia, and fibromyalgia. This resulted in data from 1032 papers at the time of our CFG analyses (634 human genetic studies, 11 human brain studies, 81 human peripheral tissue/fluids studies, 137 non-human genetic studies, 121 non-human brain studies, 48 non-human peripheral tissue/fluids studies). We have also developed a computerized CFG Wizard to automate and score large lists of genes by integrating evidence from our databases which is checked against manual scoring. Analyses were performed as previously detailed. Each gene was assigned points (Human Brain Expression Evidence – 4pts, Human Peripheral Expression Evidence – 2pts, Human Genetic Evidence - 2pts). Points were then summed with the Discovery score (0–6). Probesets/transcripts with a combined prioritization

and discovery score of at least 6 (Affymetrix study $n = 1192$, RNASeq study $n = 1697$) moved on to the next step, Validation (Fig. 1).

Step 3: Validation. For each of the separate studies, microarray and RNAseq, three groups were used for Validation Analyses: the low pain (VAS pain ≤ 2) and High Pain (VAS pain ≥ 6) groups from the Discovery cohort, along with the clinically severe subjects.

Expression levels were z-scored by gender. We carried out an ANOVA in the biomarkers that were stepwise changed in expression from low pain to high pain to clinically severe pain. Biomarkers that survived Bonferroni correction for number of biomarkers tested received 6 points, those nominally significant 4 points, and those that were just stepwise 2 points. The rest were 0 points.

Top candidate biomarkers (after the first 3 steps): Adding the scores from the first three steps into an overall convergent functional evidence (CFE) score (Fig. 1) resulted in list of top candidate biomarkers for pain that had a CFE score greater than 8 (1/3 of the possible maximum score of 24 after Step 3). These top candidate biomarkers were compared between the two studies, microarray and RNAseq, resulting in 56 biomarkers that converged and reproduced between the two (Fig. 1). These top biomarkers were carried forward for additional testing in independent cohorts (Step 4).

Step 4: Testing for clinical validity in independent cohorts. We tested in independent cohorts of patients the ability of each of the top candidate biomarkers ($n = 56$) to assess state severity (measured by VAS pain) and predict trait risk (future ER visits for pain in the first year of follow-up, and in all future years of follow-up) for patients. We conducted our analyses across all patients, as well as specifically for gender.

In each of the two studies, the test cohorts for predicting pain severity (state), and the test cohorts for predicting future ER visits with pain (trait), had housekeeping gene normalization and Z-scoring by gender. There was no subject overlap between the studies, and between the independent cohorts inside each study, as the test cohorts were independent from the discovery and validation cohorts. Individual biomarkers used for predictions were normalized as described above to avoid potential artifacts due to different ranges of expression by gene expression platform and gender. Predictions were performed using R-studio. For cross-sectional analyses, we used biomarker expression levels. For longitudinal analyses, we combined four measures: biomarker expression levels, slope (defined as the ratio of levels at current testing visit vs. previous visit, divided by time between visits), maximum levels (at any of the current or past visits), and maximum slope (between any adjacent current or past visits), as described in previous studies [12–14]. For decreased biomarkers, we used the minimum rather than the maximum for level calculations.

Predicting state-pain severity: Receiver-operating characteristic (ROC) analyses between marker levels and pain state were performed by assigning subjects' visits with a VAS pain score ≥ 6 in the high pain category vs. the remaining subjects in this independent test cohort. The Affymetrix study testing cohort consisted of 185 subjects with 462 visits, and the RNASeq study testing cohort consisted of 233 subjects with 326 visits.

Additional, chronic pain severity predictions were done in a similar manner using the SF-36 (Q21 + Q22). The Affymetrix study cohort for these predictions consisted of 185 subjects with 464 visits, and the RNASeq study cohort consisted of 223 subjects with 310 visits. A score of ≥ 10 was the cutoff for the high pain category. We used the pROC package of R (Table 1 and Fig. 1) to calculate ROC AUC and *p*-values.

Predicting trait- future ER visits due to pain as a symptom/reason: We conducted analyses for predicting future ER visits with pain as a symptom/reason for the visit in the first year following each testing visit, in subjects that had at least one year of follow-up in the VA system, in which we have access to complete electronic medical records. The Affymetrix study first-year cohort consisted of 220 subjects with 565 visits, and the RNASeq study first- year cohort consisted of 128 subjects with 227 visits. ROC analyses between biomarkers measures (cross-sectional, longitudinal) at a specific testing visit and future ER visits in the first year following testing were performed as described above, based on assigning if subjects had been admitted to the ER with pain or not in the year following testing to calculate ROC AUC and *p*-values.

We also conducted Cox regression for all future ER visits with pain, including those occurring beyond one year of follow-up (up to 18 years, average: 10.5 years). The Cox regression was performed using the time in days from visit date to first ER visit date in the case of patients who had ER visits with pain, or from visit date to last note date in the electronic medical records for those who did not. These calculations, unlike the ROC and t-test, account for the actual length of follow-up, which varied from subject to subject. The odds ratio was calculated such that a value greater than 1 always indicates increased risk for ER visits, regardless of if the biomarker is increased or decreased in expression.

Step 4 predictions scoring: Biomarkers that are nominally significant $p \leq 0.05$ (for ROC AUC for State and First Year ER visits predictions, and for Cox Regression Odds Ratio for All Future ER visits predictions) receive 3 points if they are predictive in all subjects in the cohort, and 2 points if they are only predictive within a gender. Scores are capped at 3, and the maximum score between cross-sectional and longitudinal predictions for each biomarker is taken moving forward. The best predictive biomarkers are shown in Fig. 2.

CFE4 scoring. We show in Table 1 the top biomarkers for pain ($n = 56$). For each of the two studies, the points from the 4 CFE steps are added, except that Step 2 prioritization scoring is counted only once, as it is the same in both studies. The CFE4 score is discovery + prioritization + validation + state predictions + trait predictions points, indicative of each biomarker's ability to track and predict pain (Table 1). The maximum possible CFE4 score is (6 + 12 + 6 + 12) x 2–12 = 60.

Biological understanding

Pathway analyses. DAVID Functional Annotation Analysis (National Institute of Allergy and Infectious Diseases) (v2024q1) and IPA (Ingenuity Pathway Analysis, version 111725566, Qiagen) were used to analyze the biological roles, including top canonical pathways and diseases (Table 2A, B). We performed the pathway analyses for the 56 biomarkers for pain that were the top scoring CFE biomarkers after discovery, prioritization, and validation, that converged between the two studies (Affymetrix and RNAseq).

Networks: For network analyses, we performed STRING Interaction Network (https://string-db.org) by inputting the 56 genes into the search window and performed Multiple Proteins Homo sapiens analysis (Figure S3).

CFG beyond pain: evidence for involvement in other psychiatric and related disorders: We also used a CFG approach to examine evidence from other psychiatric and related disorders, as exemplified by the list of top biomarkers (Table 1). This was not used to prioritize genes, but rather to understand the molecular basis of co-morbidities. We also looked more directly at the overlap of the top biomarkers for pain with those for other disorders previously identified by our group (Fig. 1C).

Therapeutics

Pharmacogenomics: We analyzed which of the top biomarkers for pain ($n = 56$) are known to be changed in expression by existing drugs in a direction opposite to the one in disease, using our CFG databases. These drugs and nutraceuticals are potential treatments and preventatives for patients with pain and used in the prototype reports (described below) to demonstrate personalized medicine. Drugs are also listed individually by biomarker affected (Table 1). We also calculated medication match percent based on the number of genes on our list that were targets of different drugs (Table 3A).

Drug repurposing using the connectivity map: Following biomarker identification and validation, Connectivity Map (Clue.io) was used to identify potential pharmaceuticals to alter the gene expression signature of the top biomarkers in a manner that opposes their expression in pain. A Connectivity Map Query was performed using the top pain biomarkers ($n = 56$), using L1000 parameters with the latest dataset 2.0 [17]. The results from the query were analyzed and sorted based on normalized connectivity scores and (Table 3B).

Report generation

We present examples of how reports to doctors might look, using the above insights. We selected two subjects for the reports in Fig. 3 (male: phchp479v2, female: phchp141v2) who both had clinically severe state

Table 1. Top biomarkers: convergent functional evidence (CFE).

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
CD55 CD55 Molecule (Cromer Blood Group)	201925_s_at/ ENST00000367063	(D) A/2 34.9% R/2 34.6%	8	A 0.236/2 Stepwise R 0.227/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.59/1.90E-03 **Gender-M** A VAS Pain C: (94/388) 0.59/4.86E-03 **Gender-M** A VAS Pain L: (56/234) 0.58/3.82E-02 **ALL** R VAS Pain C: (65/326) 0.57/3.55E-02		**ALL** A First-Year C: (242/565) 0.56/1.12E-02	**ALL** A Future C: (494/567) 1.13/6.63E-03 **Gender-M** A Future C: (443/507) 1.11/1.77E-02	Aging Anxiety Dementia Psychosis Stress	*American Ginseng* Carbamazepine Chlorpromazine Dexamethasone Lithium Sertraline *Sleep Deprivation* Valproate	28
PLAUR Plasminogen Activator, Urokinase Receptor	211924_s_at/ ENST00000339082	(D) A/4 55.6% R/2 34.6%	8	A 0.141/0 Not Stepwise R 0.072/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.57/4.23E-02 **Gender-M** A VAS Pain L: (56/234) 0.58/4.01E-02	**ALL** R SF-36 C: (21/310) 0.63/2.69E-02	**Gender-F** A First-Year C: (36/58) 0.64/4.32E-02	**ALL** A Future C: (494/567) 1.08/4.55E-02	Aging Alcoholism Depression Personality Disorders Stress	Brexanolone Lithium Vortioxetine	27
RIPOR2 RHO Family Interacting Cell Polarization Regulator 2	209829_at/ ENST00000644621	(D) A/4 61.9% R/4 57.7%	4	A 0.262/0 Not Stepwise R 0.422/0 Not Stepwise	**ALL** A VAS Pain C: (117/462) 0.55/4.97E-02 **ALL** R VAS Pain C: (65/326) 0.57/3.10E-02 **Gender-F** R VAS Pain C: (4/65) 0.83/1.45E-02	**ALL** R SF-36 C: (21/310) 0.66/8.68E-03 **Gender-M** R SF-36 C: (18/247) 0.64/2.18E-02	**ALL** A First-Year C: (242/565) 0.56/6.99E-03	**ALL** A Future C: (494/567) 1.1/1.57E-02 **Gender-M** A Future C: (443/507) 1.1/2.17E-02	Aging Alcoholism Dementia Depression Stress Suicidality	Clozapine Dexamethasone Vortioxetine	27

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
DENND3 DENN Domain Containing 3	212975_at/ ENST00000518668	(D) A/4 50.8% R/4 65.4%	5	A 0.064/0 Not Stepwise R 0.372/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.56/2.04E-02 **ALL** A VAS Pain L: (71/277) 0.6/6.95E-03 **Gender-M** A VAS Pain C: (94/388) 0.59/6.14E-03 **Gender-M** A VAS Pain L: (56/234) 0.6/9.29E-03		**Gender-F** A First-Year C: (36/58) 0.67/1.35E-02 **ALL** R First-Year C: (68/164) 0.6/1.61E-02 **Gender-M** R First-Year C: (49/131) 0.59/4.30E-02	**ALL** R Future C: (89/227) 1.38/1.74E-02	Aging Alcoholism Dementia Stress	Amphetamine Dexamethasone Ibuprofen Indomethacin *Magnesium* Prednisolone	26
IL1R1 Interleukin 1 Receptor Type 1	202948_at/ ENST00000409589	(D) A/4 52.4% R/4 61.5%	6	A 0.074/0 Not Stepwise R 0.515/2 Stepwise			**Gender-F** A First-Year C: (36/58) 0.78/1.76E-04 **ALL** R First-Year C: (68/164) 0.58/3.47E-02 **Gender-M** R First-Year C: (49/131) 0.6/2.26E-02	**ALL** R Future C: (89/227) 1.66/1.09E-03 **Gender-M** R Future C: (66/181) 1.79/2.97E-03	Aging Alcoholism Bipolar Dementia Depression Psychosis Stress Suicidality	Cyclosporine Ketamine Lithium Prednisolone Risperidone Tacrolimus Vortioxetine	24
IRAG1 Inositol 1,4,5-Triphosphate Receptor Associated 1	230214_at/ ENST00000547195	(D) A/2 38.1% R/2 42.3%	6	A 0.298/0 Not Stepwise R 0.108/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.58/7.10E-03 **ALL** A VAS Pain L: (71/277) 0.58/1.88E-02 **Gender-M** A VAS Pain C: (94/388) 0.57/1.75E-02 **Gender-M** A VAS Pain L: (56/234) 0.6/1.03E-02 **Gender-F** R VAS Pain C: (4/65) 0.75/4.53E-02	**ALL** R SF-36 C: (21/310) 0.63/1.95E-02 **Gender-M** R SF-36 C: (18/247) 0.64/2.42E-02	**Gender-F** A First-Year C: (36/58) 0.72/2.92E-03	**Gender-F** A Future C: (51/60) 1.54/2.93E-02	Alcoholism Bipolar Dementia Depression Stress	*Bariatric Surgery* Clozapine Ketamine Modafinil	24

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
MAPK1 Mitogen-Activated Protein Kinase 1	224621_at/ ENST00000398822	(D) A/2 38.1% R/2 34.6%	7	A 0.048/0 Not Stepwise R 0.141/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.58/7.24E-03 **Gender-M** A VAS Pain C: (94/388) 0.59/2.83E-03 **Gender-F** R VAS Pain C: (4/65) 0.77/3.58E-02	**ALL** R SF-36 C: (21/310) 0.64/1.47E-02 **Gender-M** R SF-36 C: (18/247) 0.62/4.56E-02		**ALL** A Future C: (494/567) 1.11/1.43E-02 **Gender-M** A Future C: (443/507) 1.09/3.14E-02	Aging Alcoholism Bipolar Circadian clock Dementia Depression Neurological Disorders Psychosis Stress	Antidepressants Clozapine D-cycloserine Dextromethorphan Fluoxetine Isoprenaline Lithium Nefiracetam Oxycodone Sofosbuvir Vortioxetine	24
PLCG2 Phospholipase C Gamma 2	230917_at/ ENST00000570198	(D) A/2 34.9% R/2 38.5%	6	A 0.028/0 Not Stepwise R 0.462/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.56/2.61E-02 **Gender-M** A VAS Pain C: (94/388) 0.59/5.61E-03	**ALL** R SF-36 C: (21/310) 0.66/6.12E-03 **Gender-M** R SF-36 C: (18/247) 0.66/1.17E-02	**ALL** A First-Year C: (242/565) 0.55/1.70E-02	**ALL** A Future C: (494/567) 1.11/1.32E-02 **Gender-M** A Future C: (443/507) 1.09/3.43E-02	Aging Alcoholism Bipolar Dementia Depression Psychosis Stress	Lithium *Sleep Deprivation* Valproate	24
RARA Retinoic Acid Receptor Alpha	211605_s_at/ ENST00000425707	(D) A/4 57.1% R/4 57.7%	8.5	A 0.295/0 Not Stepwise R 0.650/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.58/2.00E-02 **Gender-M** A VAS Pain L: (56/234) 0.58/3.58E-02	**Gender-F** R SF-36 C: (3/63) 0.92/7.75E-03			Aging Alcoholism Dementia Psychosis Stress Suicidality	*Bariatric Surgery* *Gamma frequency*	23.5
ANXA1 Annexin A1	201012_at/ ENST00000257497	(I) A/2 45.5% R/4 64.5%	8	A 0.141/2 Stepwise R 0.432/0 Not Stepwise	**ALL** R VAS Pain C: (65/326) 0.57/3.48E-02 **Gender-M** R VAS Pain C: (61/261) 0.58/3.81E-02	**ALL** R SF-36 C: (21/310) 0.66/7.94E-03 **Gender-M** R SF-36 C: (18/247) 0.69/3.40E-03			Aging Alcoholism ASD Bipolar Dementia Depression Neurological Disorders Psychosis Stress Suicidality	*Canova* Lithium *Magnesium* Minocycline *Omega-3 Fatty Acids*	22

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
CLK4 CDC Like Kinase 4	241403_at/ ENST00000316308	(I) A/2 40.0% R/4 61.3%	4	A 0.025/4 Stepwise R 0.053/0 Not Stepwise	**ALL** R VAS Pain C: (65/326) 0.59/1.42E-02 **Gender-M** R VAS Pain C: (61/261) 0.6/8.45E-03	**Gender-M** A SF-36 L: (3/236) 0.8/3.94E-02 **ALL** R SF-36 C: (21/310) 0.63/2.27E-02 **Gender-M** R SF-36 C: (18/247) 0.64/2.33E-02			Aging Depression Psychosis Stress Suicidality		22
CSRNP1 Cysteine And Serine Rich Nuclear Protein 1	225557_at/ ENST00000273153	(D) A/2 34.9% R/2 34.6%	8	A 0.100/0 Not Stepwise R 0.114/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.6/6.40E-03 **Gender-M** A VAS Pain C: (94/388) 0.56/3.10E-02 **Gender-M** A VAS Pain L: (56/234) 0.61/6.15E-03	**ALL** R SF-36 C: (21/310) 0.66/5.99E-03 **Gender-F** R SF-36 C: (3/63) 0.78/4.99E-02 **Gender-M** R SF-36 C: (18/247) 0.65/1.83E-02	**Gender-F** A First-Year C: (36/58) 0.63/4.94E-02		Aging Alcoholism Dementia Depression Personality Disorders Stress Suicidality	*Magnesium* Paroxetine Sertraline	22
NFE2L2 NFE2 Like BZIP Transcription Factor 2	1567013_at/ ENST00000458603	(D) A/4 52.4% R/4 53.9%	4	A 0.146/0 Not Stepwise R 0.616/0 Not Stepwise	**ALL** A VAS Pain C: (117/462) 0.55/4.02E-02 **ALL** A VAS Pain L: (71/277) 0.6/4.72E-03 **Gender-M** A VAS Pain L: (56/234) 0.6/1.41E-02 **Gender-F** R VAS Pain C: (4/65) 0.77/3.58E-02	**ALL** R SF-36 C: (21/310) 0.61/4.57E-02	**Gender-F** A First-Year C: (36/58) 0.66/2.44E-02		Alcoholism Dementia Depression Psychosis Stress Suicidality	*Acanthopanax Senticosus* Acetaminophen Antidepressants Bardoxolone *Campsis Grandiflora* Carbamazepine *Centella Asiatica* *Corydalis Yanhusuo* *Dan Zhi Herb* *Houttuynia Cordata* Ketamine *Omega-3 Fatty Acids* *Psoralea Corylifolia* *Quercetin* *Valeriana Officinalis* *Withaniasomnifera*	22

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
RHOA Ras Homolog Family Member A	1555814_a_at/ ENST00000676712	(D) A/2 41.3% R/4 61.5%	4	A 0.091/2 Stepwise R 0.673/2 Stepwise	**Gender-F** A VAS Pain C: (23/74) 0.65/2.26E-02 **ALL** R VAS Pain C: (65/326) 0.57/4.35E-02	**ALL** R SF-36 C: (21/310) 0.64/1.78E-02 **Gender-M** R SF-36 C: (18/247) 0.64/2.82E-02			Alcoholism ASD Dementia Depression Psychosis Stress Suicidality	Lithium	22
CTSB Cathepsin B	213275_x_at/ ENST00000531551	(D) A/4 63.5% R/4 53.9%	8	A 0.434/0 Not Stepwise R 0.074/0 Not Stepwise			**Gender-M** R First-Year C: (49/131) 0.61/1.88E-02	**ALL** R Future C: (89/227) 1.35/2.15E-02 **Gender-M** R Future C: (66/181) 1.48/2.00E-02	Aging Alcoholism Anxiety Bipolar Dementia Depression Other Addictions Psychosis Stress	Carbamazepine Clozapine Ketamine Lithium Mianserin *Omega-3 Fatty Acids* Valproate	21
GZMK Granzyme K	206666_at/ ENST00000231009	(I) A/2 38.2% R/2 38.7%	8	A 0.820/0 Not Stepwise R 0.371/0 Not Stepwise	**ALL** R VAS Pain C: (65/326) 0.59/1.33E-02 **Gender-M** R VAS Pain C: (61/261) 0.6/7.93E-03		**ALL** A First-Year C: (242/565) 0.56/7.53E-03	**ALL** A Future C: (494/567) 1.08/3.19E-02 **Gender-M** A Future C: (443/507) 1.08/3.76E-02	Anxiety Stress		21
MAEA Macrophage Erythroblast Attacher, E3 Ubiquitin Ligase	207922_s_at/ ENST00000264750	(D) A/4 76.2% R/4 65.4%	4	A 0.007/0 Not Stepwise R 0.704/0 Not Stepwise	**ALL** A VAS Pain C: (117/462) 0.56/3.69E-02	**ALL** R SF-36 C: (21/310) 0.66/8.45E-03 **Gender-F** R SF-36 C: (3/63) 0.79/4.66E-02 **Gender-M** R SF-36 C: (18/247) 0.64/2.62E-02		**ALL** A Future C: (494/567) 1.11/1.52E-02 **Gender-M** A Future C: (443/507) 1.1/2.30E-02	Aging Alcoholism Anxiety Bipolar Dementia Depression Psychosis Stress	Prednisolone *S-adenosyl methionine*	21
RPL31 Ribosomal Protein L31	241017_at/ ENST00000409320	(I) A/2 38.2% R/4 58.1%	4	A 0.228/2 Stepwise R 0.258/0 Not Stepwise	**ALL** R VAS Pain C: (65/326) 0.58/2.15E-02 **Gender-M** R VAS Pain C: (61/261) 0.59/2.04E-02	**ALL** R SF-36 C: (21/310) 0.64/1.38E-02 **Gender-M** R SF-36 C: (18/247) 0.67/7.86E-03	**ALL** R First-Year C: (68/164) 0.59/2.02E-02 **Gender-M** R First-Year C: (49/131) 0.61/1.80E-02		Alcoholism Bipolar Dementia Depression Psychosis Stress Suicidality	Lithium Dexamethasone Ketamine	21

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
RPS24 Ribosomal Protein S24	1555878_at/ ENST00000372360	(I) A/2 47.3% R/4 51.6%	4	A 0.551/2 Stepwise R 0.191/0 Not Stepwise	**ALL** R VAS Pain C: (65/326) 0.57/4.94E-02 **Gender-M** R VAS Pain C: (61/261) 0.57/4.37E-02	**ALL** R SF-36 C: (21/310) 0.65/1.08E-02 **Gender-M** R SF-36 C: (18/247) 0.7/2.45E-03	**ALL** R First-Year C: (68/164) 0.58/4.53E-02 **Gender-M** R First-Year C: (49/131) 0.59/4.00E-02		Alcoholism Dementia Depression Other Addictions Psychosis Stress	Citalopram	21
RPSA Ribosomal Protein SA	213801_x_at/ ENST00000301821	(I) A/2 47.3% R/4 51.6%	8	A 0.056/2 Stepwise R 0.575/0 Not Stepwise		**ALL** R SF-36 C: (21/310) 0.63/1.95E-02 **Gender-M** R SF-36 C: (18/247) 0.63/3.52E-02	**Gender-M** A First-Year C: (206/507) 0.56/1.26E-02		Alcoholism Bipolar Dementia Depression Other Addictions Psychosis	Dexamethasone	21
TSC22D4 TSC22 Domain Family Member 4	1554501_at/ ENST00000393991	(D) A/2 36.5% R/4 61.5%	8	A 0.144/0 Not Stepwise R 0.863/2 Stepwise	**Gender-F** R VAS Pain C: (4/65) 0.82/1.66E-02		**Gender-F** A First-Year C: (36/58) 0.69/7.43E-03		Alcoholism Dementia Depression Stress	Estradiol	20
GLUL Glutamate-Ammonia Ligase	217202_s_at/ ENST00000417584	(D) A/2 41.3% R/2 42.3%	8	A 0.862/2 Stepwise R 0.461/2 Stepwise		**ALL** R SF-36 C: (21/310) 0.66/7.72E-03 **Gender-M** R SF-36 C: (18/247) 0.65/1.53E-02			Alcoholism Anxiety Bipolar **Circadian clock** Dementia Depression Other Addictions Psychosis Stress Suicidality	Ketamine Lithium Nortriptyline *Omega-3 Fatty Acids* Paroxetine Prednisolone Vortioxetine	19
ILRUN Inflammation And Lipid Regulator With UBA-Like And NBR1-Like Domains	217924_at/ ENST00000374026	(D) A/4 54.0% R/4 53.9%	6	A 0.302/0 Not Stepwise R 0.444/2 Stepwise		**ALL** R SF-36 C: (21/310) 0.65/1.31E-02 **Gender-F** R SF-36 C: (3/63) 0.88/1.41E-02			Aging Anxiety Bipolar Dementia Depression Psychosis Stress Suicidality	Lithium	19

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
LILRB3 Leukocyte Immunoglobulin Like Receptor B3	211133_x_at/ ENST00000436504	(D) A/2 33.3% R/2 42.3%	10	A 0.100/0 Not Stepwise R 0.172/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.56/2.48E-02 **ALL** A VAS Pain L: (71/277) 0.6/6.13E-03 **Gender-M** A VAS Pain C: (94/388) 0.57/2.58E-02 **Gender-M** A VAS Pain L: (56/234) 0.61/7.58E-03				Dementia Depression Stress	Fluoxetine	19
MIDN Midnolin	225954_s_at/ ENST00000300952	(D) A/2 41.3% R/2 46.2%	8	A 0.019/0 Not Stepwise R 0.303/2 Stepwise	**Gender-F** R VAS Pain C: (4/65) 0.75/4.79E-02	**ALL** R SF-36 C: (21/310) 0.63/2.37E-02			Aging Alcoholism Depression Psychosis Stress Suicidality	Amphetamine Ketamine *Nicotinamide Riboside* *S-adenosyl methionine*	19
RFX2 Regulatory Factor X2	226872_at/ ENST00000303657	(D) A/4 57.1% R/4 53.9%	4	A 0.011/0 Not Stepwise R 0.098/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.57/3.09E-02 **Gender-M** A VAS Pain L: (56/234) 0.58/3.73E-02	**Gender-F** R SF-36 C: (3/63) 0.83/2.84E-02			Alcoholism Depression Psychosis Stress	Ketamine Lithium *Omega-3 Fatty Acids* Valproate	19
SNCA Synuclein Alpha	207827_x_at/ ENST00000508895	(D) A/4 68.3% R/4 69.2%	6	A 0.898/0 Not Stepwise R 0.219/0 Not Stepwise			**ALL** R First-Year C: (68/164) 0.59/1.97E-02 **Gender-M** R First-Year C: (49/131) 0.61/2.21E-02	**Gender-M** R Future C: (66/181) 1.55/3.68E-02	Aging Alcoholism Anxiety Bipolar Dementia Depression Neurological Disorders Other Addictions Psychosis Stress Suicidality	Antipsychotics Lithium Nortriptyline *Omega-3 Fatty Acids* Valproate Vortioxetine	19

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
WASF2 WASP Family Member 2	224563_at/ ENST00000618852	(D) A/4 57.1% R/2 38.5%	6	A 0.169/0 Not Stepwise R 0.250/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.57/4.34E-02 **Gender-M** A VAS Pain L: (56/234) 0.58/3.58E-02 **Gender-F** R VAS Pain C: (4/65) 0.75/4.53E-02				Alcoholism Bipolar Dementia Depression Psychosis	Amphetamine Lithium *Magnesium* *S-adenosyl methionine* Vortioxetine	19
CCNL2 Cyclin L2	232274_at/ ENST00000480479	(D) A/2 38.1% R/2 38.5%	6	A 0.888/0 Not Stepwise R 0.942/0 Not Stepwise	**Gender-M** A VAS Pain L: (56/234) 0.58/4.48E-02		**ALL** R First-Year C: (68/164) 0.6/1.54E-02 **Gender-F** R First-Year C: (19/33) 0.72/1.73E-02	**ALL** R Future C: (89/227) 1.37/3.85E-02 **Gender-F** R Future C: (23/46) 1.63/4.20E-02	Aging Alcoholism Anxiety Dementia Other Addictions Stress	Ketamine *Omega-3 Fatty Acids*	18
CUX1 Cut Like Homeobox 1	214743_at/ ENST00000622516	(D) A/4 60.3% R/4 50.0%	6	A 0.011/0 Not Stepwise R 0.146/2 Stepwise	**Gender-F** R VAS Pain C: (4/65) 0.75/4.79E-02				Aging Alcoholism Dementia Depression Psychosis Stress	Clozapine Dexamethasone *Omega-3 Fatty Acids* Paroxetine Prednisolone Risperidone	18
MBNL3 Muscleblind Like Splicing Regulator 3	219814_at/ ENST00000370853	(D) A/4 58.7% R/4 53.9%	4	A 0.541/0 Not Stepwise R 0.372/0 Not Stepwise			**ALL** R First-Year C: (68/164) 0.61/7.69E-03 **Gender-M** R First-Year C: (49/131) 0.62/1.09E-02	**ALL** R Future C: (89/227) 1.29/4.61E-02	ADHD Alcoholism Anxiety Dementia Psychosis	Brexanolone *Magnesium* *Nicotinamide Riboside* Prednisolone Valproate	18
RPL13A Ribosomal Protein L13a	200715_x_at/ ENST00000676477	(I) A/2 40.0% R/2 45.2%	6	A 0.273/2 Stepwise R 0.334/0 Not Stepwise		**ALL** R SF-36 C: (21/310) 0.62/3.35E-02 **Gender-M** R SF-36 C: (18/247) 0.63/2.91E-02	**ALL** A First-Year C: (242/565) 0.54/4.86E-02		Alcoholism Anxiety Dementia Depression Other Addictions Psychosis Suicidality	Cyclosporine Lithium *Omega-3 Fatty Acids*	18

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
BANP BTG3 Associated Nuclear Protein	219966_x_at/ ENST00000612301	(D) A/4 54.0% R/2 34.6%	6	A 0.135/0 Not Stepwise R 0.869/0 Not Stepwise	**ALL** A VAS Pain L: (71/277) 0.57/4.35E-02 **Gender-M** A VAS Pain C: (94/388) 0.57/2.06E-02 **Gender-M** A VAS Pain L: (56/234) 0.58/3.84E-02		**Gender-F** A First-Year C: (36/58) 0.65/2.53E-02		Alcoholism Dementia Depression Other Addictions Stress	*Magnesium*	17
FUT7 Fucosyltransferase 7	217696_at/ ENST00000314412	(D) A/2 42.9% R/2 34.6%	4	A 0.571/2 Stepwise R 0.243/2 Stepwise	**Gender-M** A VAS Pain L: (56/234) 0.58/3.67E-02	**ALL** R SF-36 C: (21/310) 0.65/9.68E-03 **Gender-M** R SF-36 C: (18/247) 0.64/2.46E-02			Aging Alcoholism Depression Other Addictions Psychosis Suicidality	Haloperidol Imipramine Ketamine Prednisolone Vortioxetine	17
MAP2K7 Mitogen-Activated Protein Kinase Kinase 7	216206_x_at/ ENST00000397979	(D) A/4 54.0% R/4 57.7%	4	A 0.377/0 Not Stepwise R 0.218/2 Stepwise		**ALL** R SF-36 C: (21/310) 0.61/5.00E-02			Aging Alcoholism ASD Depression Other Addictions Psychosis Stress Suicidality		17
RNF10 Ring Finger Protein 10	207801_s_at/ ENST00000542701	(D) A/4 58.7% R/4 53.9%	2	A 0.849/2 Stepwise R 0.879/2 Stepwise				**ALL** R Future C: (89/227) 1.34/2.02E-02 **Gender-M** R Future C: (66/181) 1.34/4.31E-02	Aging Alcoholism Depression Psychosis	Clozapine Ketamine Lithium *Omega-3 Fatty Acids*	17
CEP57 Centrosomal Protein 57	203493_s_at/ ENST00000541150	(I) A/2 34.6% R/4 58.1%	6	A 0.771/2 Stepwise R 0.328/0 Not Stepwise		**Gender-M** R SF-36 C: (18/247) 0.62/4.00E-02			Aging Alcoholism Bipolar Dementia Depression Psychosis Stress	*Celastrol Magnesium* Valproate	16
GIT2 GIT ArfGAP 2	204982_at/ ENST00000457474	(D) A/4 50.8% R/2 42.3%	5	A 0.043/0 Not Stepwise R 0.376/2 Stepwise	**ALL** A VAS Pain C: (117/462) 0.59/2.83E-03 **Gender-M** A VAS Pain C: (94/388) 0.58/6.61E-03				Aging Alcoholism Dementia Psychosis Stress Suicidality	*Magnesium*	16

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
MICAL2 Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2	243611_at/ ENST00000530691	(D) A/4 68.3% R/2 38.5%	6	A 0.395/0 Not Stepwise R 0.566/2 Stepwise		**Gender-F** R SF-36 C: (3/63) 0.88/1.41E-02			Alcoholism Anxiety ASD Bipolar Dementia Depression Mood Psychosis Stress Suicidality	Amphetamine Carbamazepine Clozapine Lithium	16
CD52 CD52 Molecule	204661_at/ ENST00000374213	(I) A/2 45.5% R/4 51.6%	6	A 0.271/0 Not Stepwise R 0.375/0 Not Stepwise		**ALL** R SF-36 C: (21/310) 0.62/3.48E-02 **Gender-M** R SF-36 C: (18/247) 0.63/3.09E-02			Alcoholism Appetite Dementia Depression Psychosis Stress	Dexamethasone Escitalopram Indomethacin	15
KDM4B Lysine Demethylase 4B	212492_s_at/ ENST00000588361	(D) A/4 61.9% R/2 34.6%	5	A 0.02/0 Not Stepwise R 0.426/2 Stepwise			**Gender-F** A First-Year C: (36/58) 0.75/8.42E-04		Aging Alcoholism Bipolar Dementia Depression Stress Suicidality	Ketamine Lithium *Magnesium* *Omega-3 Fatty Acids*	15
MALAT1 Metastasis Associated Lung Adenocarcinoma Transcript 1	224559_at/ ENST00000710849	(I) A/4 52.7% R/2 38.7%	6	A 0.065/0 Not Stepwise R 0.245/0 Not Stepwise		**ALL** R SF-36 C: (21/310) 0.63/2.47E-02 **Gender-M** R SF-36 C: (18/247) 0.62/4.00E-02			Aging Alcoholism Anxiety Bipolar Dementia Depression Other Addictions Psychosis Stress Suicidality	Clozapine *Gamma frequency* Indomethacin Lithium Memantine *Omega-3 Fatty Acids* Resveratrol Sertraline	15
NOTCH1 Notch Receptor 1	218902_at/ ENST00000680003	(D) A/2 38.1% R/4 50.0%	6	A 0.112/0 Not Stepwise R 0.868/0 Not Stepwise			**ALL** R First-Year C: (68/164) 0.59/2.75E-02		Aging Alcoholism Anxiety Bipolar Dementia Depression Psychosis Stress Suicidality	Amphetamine *Bariatric Surgery* Fluvoxamine Lithium *S-adenosine methionine*	15
RNF123 Ring Finger Protein 123	224186_s_at/ ENST00000498376	(D) A/2 36.5% R/4 57.7%	6	A 0.714/0 Not Stepwise R 0.684/0 Not Stepwise		**ALL** R SF-36 C: (21/310) 0.62/3.50E-02 **Gender-F** R SF-36 C: (3/63) 0.89/1.19E-02			Aging Alcoholism Bipolar Depression Psychosis Stress Suicidality	*Omega-3 Fatty Acids*	15

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
SEMA4A Semaphorin 4A	219259_at/ ENST00000355014	(D) A/4 57.1% R/2 46.2%	4	A 0.067/0 Not Stepwise R 0.329/2 Stepwise	**ALL** A VAS Pain L: (71/277) 0.57/4.18E-02 **Gender-M** A VAS Pain L: (56/234) 0.59/2.68E-02				Aging Alcoholism ASD Depression Psychosis Stress	Dexamethasone	15
ELF4 E74 Like ETS Transcription Factor 4	31845_at/ ENST00000335997	(D) A/2 41.3% R/2 34.6%	6	A 0.191/0 Not Stepwise R 0.096/2 Stepwise	**Gender-F** R VAS Pain C: (4/65) 0.77/3.37E-02				Aging Alcoholism Dementia Psychosis Stress	Carbamazepine	14
HES1 Hes Family BHLH Transcription Factor 1	203395_s_at/ ENST00000232424	(D) A/4 52.4% R/2 34.6%	6	A 0.566/0 Not Stepwise R 0.915/0 Not Stepwise		**Gender-F** R SF-36 C: (3/63) 0.81/3.79E-02			Aging Alcoholism Anxiety Depression Mood Psychosis Sleep Disorders Stress Suicidality	Amphetamine Fluvoxamine *Magnesium*	14
SLC6A8 Solute Carrier Family 6 Member 8	202219_at/ ENST00000253122	(D) A/2 42.9% R/6 88.5%	4	A 0.909/2 Stepwise R 0.158/0 Not Stepwise					Aging Alcoholism ASD Depression Stress Suicidality	Antipsychotics Carbamazepine Estradiol Lithium	14
YBX3 Y-Box Binding Protein 3	201160_s_at/ ENST00000279550	(D) A/2 36.5% R/6 84.6%	6	A 0.877/0 Not Stepwise R 0.071/0 Not Stepwise					Aging Alcoholism Anxiety Bipolar Depression Psychosis Stress Suicidality	*American Ginseng* Antipsychotics Benzodiazepines Lithium *Meditation* Mianserin	14
NFKB1 Nuclear Factor Kappa B Subunit 1	209239_at/ ENST00000652569	(D) A/4 52.4% R/2 34.6%	5.5	A 0.076/0 Not Stepwise R 0.664/2 Stepwise					Aging Alcoholism Anxiety Dementia Depression Psychosis Stress Suicidality	Desipramine Fluoxetine *Helicid* *Melatonin* Metformin Methotrexate Olanzapine Vortioxetine	13.5
HLA-B Major Histocompatibility Complex, Class I, B	208729_x_at/ ENST00000696558	(D) A/2 42.9% R/2 38.5%	6	A 0.358/0 Not Stepwise R 0.274/2 Stepwise					Bipolar Dementia Depression Psychosis Sleep Disorders Stress Suicidality	*Gamma frequency* Lithium *Omega-3 fatty acids* Sertraline Valproate	12

Table 1. continued

Gene Symbol/Name	Probesets/ Transcript	Step 1 Discovery (Direction of Change in Pain) Platform/ Score/%	Step 2 Prioritization Convergent Functional Genomics (CFG) Evidence for Involvement in Pain Score	Step 3 Validation ANOVA *p*-value/ Score	Step 4 Significant Prediction of VAS Pain ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Prediction of Chronic Pain SF-36 ROC AUC/ *p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of First Year ER visits for Pain ROC AUC/*p*-value All 3 pt. M/F 2 pt	Step 4 Significant Predictions of Future ER Visits for Pain OR/OR *p*-value All 3 pt. M/F 2 pt	Other Psychiatric and Related Disorders Evidence	Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1–4)
MARCHF8 Membrane Associated Ring-CH-Type Finger 8	221824_s_at/ ENST00000453424	(D) A/4 54.0% R/4 61.5%	4	A 0.682/0 Not Stepwise R 0.466/0 Not Stepwise					Aging Alcoholism Dementia Depression Other Addictions Psychosis Stress	Fluoxetine *Omega-3 Fatty Acids*	12
MKRN1 Makorin Ring Finger Protein 1	201285_at/ ENST00000475010	(D) A/4 57.1% R/2 46.2%	4	A 0.977/0 Not Stepwise R 0.296/2 Stepwise					Alcoholism Anxiety Dementia Depression Other Addictions Psychosis Stress Suicidality	Clozapine *Omega-3 Fatty Acids* Paroxetine Valproate	12
TRIM58 Tripartite Motif Containing 58	215047_at/ ENST00000366481	(D) A/4 74.6% R/4 69.2%	4	A 0.797/0 Not Stepwise R 0.227/0 Not Stepwise					Psychosis Stress Suicidality	Antipsychotics *Meditation* Prednisolone *S-adenosyl methionine*	12
CRY1 Cryptochrome Circadian Regulator 1	209674_at/ ENST00000008527	(I) A/2 40.0% R/2 38.7%	7	A 0.802/0 Not Stepwise R 0.062/0 Not Stepwise					Aging Alcoholism Anxiety Bipolar Circadian clock Depression Stress	Aripiprazole Fluoxetine Ketamine Lithium Risperidone *Sea cucumber saponin*	11
APBA3 Amyloid Beta Precursor Protein Binding Family A Member 3	215148_s_at/ ENST00000316757	(D) A/2 33.3% R/2 34.6%	6	A 0.391/0 Not Stepwise R 0.351/0 Not Stepwise					Aging Dementia Depression Psychosis Stress	Vortioxetine	10

Biomarkers ($n = 56$) that were in common from the two platforms after Step 3 (**A**-Affymetrix and **R**-RNASeq).
For Step 4 Predictions, ***C*** cross-sectional (using levels from one visit), ***L*** longitudinal (using levels and slopes from multiple visits). In **ALL**, and by Gender. ***M*** Males, ***F*** Females. For Step 4 predictions scoring, 3 pts if significant in all, 2 points if by gender. Capped at 3 from each platform, for each phenotype predicted. *Italic- nutraceuticals/alternative treatments*.

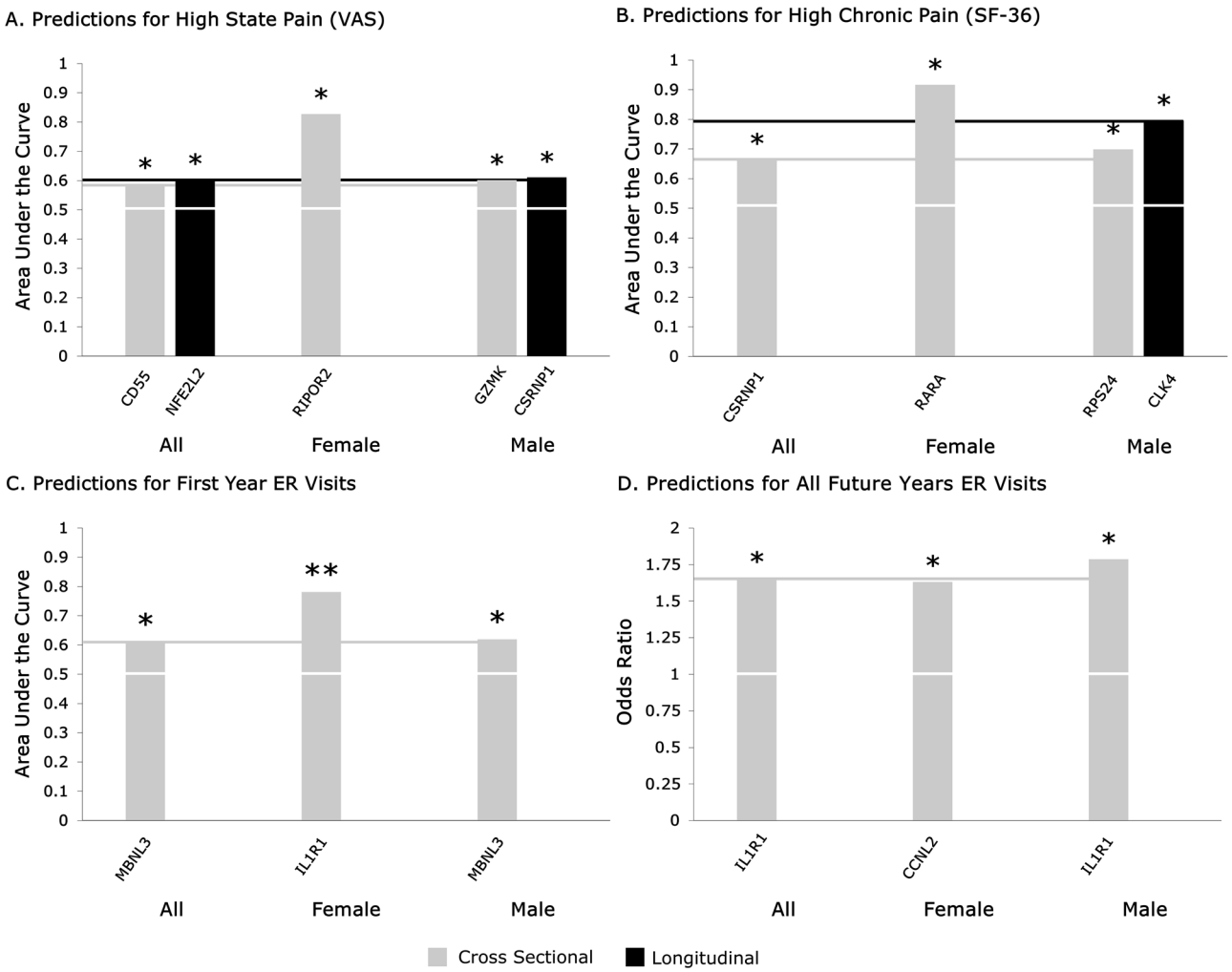


Fig. 2 Best individual biomarkers predictors. Top cross-sectional and longitudinal markers are shown in all subjects, males, and females. **A**. Predictions for High State Pain (VAS). **B**. Predictions for High Chronic Pain (SF-36- Q21 + Q22). **C**. Predictions for First Year ER Visits **D**. Predictions for All Future Years ER Visits. * Nominally significant. ** Bonferroni significant after correcting for 56 biomarkers tested.

and chronic pain (VAS pain ≥ 6, SF-36 Q21 of 6, SF-36 Q22 of 5), as well as psychiatric and co-morbid pain diagnoses (chronic low back pain for the major depressive disorder male, fibromyalgia for the bipolar female). We used a panel of the top predictive biomarkers for state and ER visits within the first year, by gender.

Step 5 – Generalizability. Top biomarkers for pain disorders ($n = 56$) were retested for predictive ability on the whole database ($n = 1186$), consisting of males ($n = 942$) and females ($n = 244$). The raw expression values of the biomarkers in our whole gene expression dataset ($n = 1186$) were Z-scored by gender and by platform (e.g. RNASeq or microarray) in order to be able to combine them. Subsequently, to create our biomarker panel for the reports, for each gender we took the 12 best predictive biomarkers for state, and the 13 best predictive biomarkers for first year ER visits, resulting in a male and female panel of 25 biomarkers each.

Score generation: For state score, the Z-scored expression value of each increased biomarker was compared to the average value for the biomarker in the high pain group in the database, resulting in scores of 1 or 0 respectively, and 0.5 if it is in between. The reverse was done for decreased biomarkers.

For trait future risk score, we calculated the average expression value for a biomarker in the first-year ER visits for pain group, and subjects with no ER visits for pain in the first-year group. We then compared the biomarkers for the subject of interest to these reference levels. If a biomarker was higher than the average of the high group it got a 1, if it was below the average of the no group it got a 0, and if it was in between, it got a 0.5 for increased biomarkers. For decreased biomarkers, if it was lower than the average of the high group it got a 1, if it was higher than the average of the no group it got a 0, and if it was in between it got a 0.5. These digitized scores for each biomarker are multiplied by the CFE4 score of each biomarker as a weight, to account for the totality of evidence, then summed into a polygenic risk score and then divided by a sum of all CFE4 scores.

The pain state risk score is the average score of all the state biomarkers multiplied by 100, generating 3 risk categories: high (red), intermediate (yellow), and low (green). The chronic pain risk score was calculated the same way using biomarkers for first year ER visits due to pain. These percentile scores of the patient are provided in the report (Fig. 3). The digitized biomarkers are also used for matching with existing psychiatric medications and alternative treatments (nutraceuticals and others). We use our large datasets and literature databases to match biomarkers to medications that have effects on gene expression opposite to their expression in high pain. The gene expression data is from gene expression data in human and animal models. Each medication matched to a biomarker gets the biomarker's score of 1, 0.5 or 0. The scores for the medications are added, and divided by the number of biomarkers that were 1 or 0.5 in that patient, resulting in a percentile match. Thus, psychiatric medications are matched to the patient and ranked in order of impact on the panel.

RESULTS

In Step 1 Discovery, we used a powerful within –subject and then across-subject design in a longitudinally followed cohort of

Table 2. Biological analyses. A. Pathways. B. Diseases. C. Upstream Regulators.

2A.	**DAVID Functional Annotation Biological Processes**					**Ingenuity Pathways**		
	#	**Term**	**Count**	**%**	***P*-Value**	**Top Canonical Pathways**	***P*-Value**	**Overlap**
Top CFE BioM ($n = 56$ genes)	1	**cellular response to tumor necrosis factor**	5	8.9	6.31E-04	**Neuroinflammation Signaling Pathway**	6.39E-07	2.5% 8/317
	2	cytoplasmic translation	4	7.1	2.17E-03	Neutrophil degranulation	1.28E-05	1.7% 8/476
	3	regulation of transcription from RNA polymerase II promoter	12	21.4	4.95E-03	Regulation of NFE2L2	1.57E-05	66.7% 2/3
	4	response to interleukin-1	3	5.4	5.00E-03	HMGB1 Signaling	4.18E-05	3.0% 5/167
	5	negative regulation of transcription from RNA polymerase II promoter	9	16.1	7.62E-03	PEDF Signaling	4.27E-05	4.8% 4/84
2B.	**David Genetic Association Disease**					**Ingenuity Pathways Disease**		
	#	**Term**	**Count**	**%**	***P*-Value**	**Diseases and Disorders**	***P*-Value**	**# Molecules**
Top CFE BioM ($n = 56$ genes)	1	**ankylosing spondylitis**	3	5.4	9.34E-03	**Inflammatory Response**	2.33E-03–9.97E-11	33
	2	Graft vs Host Disease|Hematologic Diseases	2	3.6	1.93E-02	Endocrine System Disorders	2.31E-03–3.75E-09	55
	3	**arthritis, psoriatic**	2	3.6	1.93E-02	Gastrointestinal Disease	2.31E-03–3.75E-09	55
	4	Chorioamnionitis|Fetal Membranes, Premature Rupture|Infection of amniotic sac and membranes	4	7.1	2.23E-02	Metabolic Disease	2.31E-03–3.75E-09	27
	5	Hemorrhagic Fever with Renal Syndrome	2	3.6	2.25E-02	Organismal Injury and Abnormalities	2.34E-03–3.75E-09	56

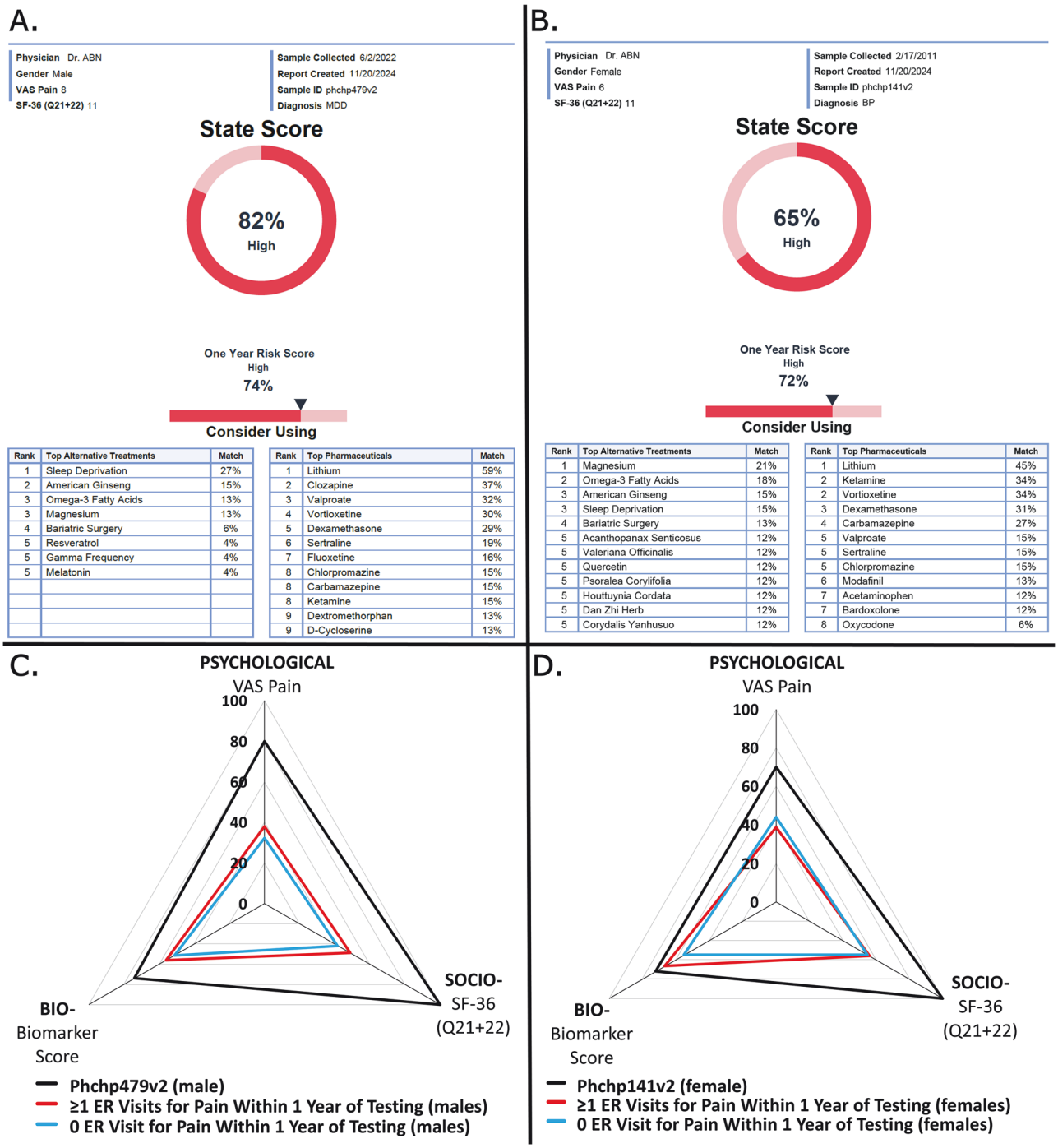
2C. Upstream Regulators Top CFE BioM	
Regulator	***P*-Value**
TNF	1.50E–08
IL1B	2.89E-08
MYC	2.01E-07
D-Glucose	9.52E-07
ESR1	1.89E-06

Table 3. Treatments **A. Matching with treatments**. Using our literature databases of drugs that have opposite effects on the gene expression signature of top biomarkers from Table 1. **B. Drug Repurposing**. Using Connectivity Map analysis of drugs that have opposite effects on the gene expression signature of top biomarkers from Table 1. **Bold-known treatments for pain**. *Italic-Nutraceutical/Alternative treatments*.

A. Treatments	Percentile
Lithium	42.86%
Omega-3 Fatty Acids	*28.57%*
Ketamine	**23.21%**
Magnesium	*17.86%*
Vortioxetine	17.86%
Clozapine	16.07%
Valproate	16.07%
Dexamethasone	**14.29%**
Prednisolone	**14.29%**
Amphetamine	10.71%
Carbamazepine	**10.71%**
Fluoxetine	8.93%
S-adenosyl methionine	*8.93%*
Paroxetine	7.14%
Sertraline	7.14%
Bariatric Surgery	*5.36%*
Gamma frequency	*5.36%*
Indomethacin	**5.36%**
Risperidone	5.36%
American Ginseng	*3.57%*
Brexanolone	3.57%
Cyclosporine	3.57%
Estradiol	3.57%
Fluvoxamine	3.57%
Meditation	*3.57%*
Mianserin	3.57%
Nicotinamide Riboside	*3.57%*
Nortriptyline	**3.57%**
Sleep Deprivation	*3.57%*
Acetaminophen	**1.79%**
Desipramine	**1.79%**
Dextromethorphan	1.79%
Escitalopram	1.79%
Haloperidol	1.79%
Ibuprofen	**1.79%**

Table 3. continued

B. Repurposed Drug	Connectivity Score	Mechanisms of action
B. Repurposed Drug	Connectivity Score	Mechanisms of action
Thalidomide	1.68	TNF inhibitor
Carvedilol	1.67	Adrenergic receptor antagonist
Amyleine	1.66	Anesthetic - local
Sirolimus	1.66	Immunosupresor-MTOR inhibitor
Budesonide	1.66	Steroid
Berbamine	*1.65*	*Calmodulin inhibitor*
Sulindac	**1.64**	**Cyclooxygenase inhibitor**
Quetiapine	1.63	Dopamine receptor antagonist
Bazedoxifene	1.62	Selective estrogen receptor modulator
Sufentanil	**1.62**	**Opioid receptor modulator**
Edaravone	1.62	Nootropic agent
Carbamazepine	**1.61**	**Anticonvulsant**
Meclofenamic-Acid	**1.56**	**Cyclooxygenase inhibitor**
Meloxicam	**1.56**	**Cyclooxygenase inhibitor**
Inositol	*1.55*	*Insulin sensitizer*
Oxcarbazepine	**1.54**	**Sodium channel inhibitor**
Zolpidem	1.54	Benzodiazepine receptor agonist
Milnacipran	**1.52**	**Serotonin receptor antagonist**
Lorazepam	1.51	Benzodiazepine receptor agonist
Rofecoxib	**1.51**	**Cyclooxygenase inhibitor**
Nimesulide	**1.50**	**Cyclooxygenase inhibitor**
Prednisolone	1.50	Steroid
Naringenin	*1.50*	*Aromatase inhibitor|TRPV antagonist*
Curcumin	*1.50*	*Spice*
Capsazepine	1.50	TRPV agonist
Pregnenolone	1.49	Neurohormone
Mianserin	1.49	Serotonin receptor antagonist
Tramadol	**1.49**	**Opioid agonist**
Sotalol	1.45	Adrenergic receptor antagonist
Luteolin	*1.44*	*Glucosidase inhibitor*
Pindolol	1.44	Adrenergic receptor antagonist
Quercetin	*1.44*	*Polar auxin transport inhibitor*
Piroxicam	**1.44**	**Cyclooxygenase inhibitor**
Noscapine	**1.44**	**Opioid agonist**
Biotin	*1.43*	*B vitamin*
Mesalazine	**1.43**	**Cyclooxygenase inhibitor**
Duloxetine	**1.43**	**SNRI**



Rank	Top Alternative Treatments	Match
1	Sleep Deprivation	27%
2	American Ginseng	15%
3	Omega-3 Fatty Acids	13%
3	Magnesium	13%
4	Bariatric Surgery	6%
5	Resveratrol	4%
5	Gamma Frequency	4%
5	Melatonin	4%

Rank	Top Pharmaceuticals	Match
1	Lithium	59%
2	Clozapine	37%
3	Valproate	32%
4	Vortioxetine	30%
5	Dexamethasone	29%
6	Sertraline	19%
7	Fluoxetine	16%
8	Chlorpromazine	15%
8	Carbamazepine	15%
8	Ketamine	15%
9	Dextromethorphan	13%
9	D-Cycloserine	13%

Rank	Top Alternative Treatments	Match
1	Magnesium	21%
2	Omega-3 Fatty Acids	18%
3	American Ginseng	15%
3	Sleep Deprivation	15%
4	Bariatric Surgery	13%
5	Acanthopanax Senticosus	12%
5	Valeriana Officinalis	12%
5	Quercetin	12%
5	Psoralea Corylifolia	12%
5	Houttuynia Cordata	12%
5	Dan Zhi Herb	12%
5	Corydalis Yanhusuo	12%

Rank	Top Pharmaceuticals	Match
1	Lithium	45%
2	Ketamine	34%
2	Vortioxetine	34%
3	Dexamethasone	31%
4	Carbamazepine	27%
5	Valproate	15%
5	Sertraline	15%
5	Chlorpromazine	15%
6	Modafinil	13%
7	Acetaminophen	12%
7	Bardoxolone	12%
8	Oxycodone	6%

Fig. 3 Individual patient reports and radar plot. A. Male. **B**. Female. Reports based on panels of top predictive biomarkers for that gender. **C**, **D**. Radar plots showing the two subjects biomarker panel score and clinical measures, compared to averages in the whole cohort we studied.

subjects who displayed at least a change in the pain measure (from VAS 2 and below to 6 and above, and vice-versa) between at least two consecutive testing visits, to identify differentially expressed genes that track pain state.

In Step 2 Prioritization, we used a Convergent Functional Genomics (CFG) approach to prioritize the candidate biomarkers identified in the discovery step (33% cutoff/internal score of ≥ 2 pt.) by using prior published literature evidence (genetic, gene expression and proteomic), from human and animal model studies, for involvement in pain (Fig. 1 and Table 1 and S2). Probesets/ transcripts that had a total score (combined discovery score and prioritization score) of 6 and above were carried forward to the validation step.

In Step 3 Validation, we validated the prioritized candidate biomarkers for change in an independent cohort of patients with clinically severe chronic pain as measured by items 21 and 22 from SF-36 (Figure S1B). We assessed which biomarkers were stepwise changed in expression from low pain in the discovery cohort to high pain in the discovery cohort, to clinically severe pain (Fig. 1).

Adding the scores from the first three steps into an overall convergent functional evidence (CFE) score (Fig. 1), we ended up with a list of 659 top candidate biomarkers for pain from the microarray

samples cohorts, and 687 top candidate biomarkers for pain from the RNAseq samples cohorts, that had a CFE3 score ≥ 8, better than 33% of the maximum possible score of 24 after the first three steps, which we decided to use as an empirical cutoff. 56 biomarkers were in common between the two lists, so were reproducible across multiple independent cohorts and different gene expression platforms. These top candidate biomarkers were then tested in Step 4 for clinical validity/predictive ability in additional independent cohorts, on the two respective platforms (Fig. 1 and Table 1).

Testing for clinical validity

For each platform (Affymetrix, RNAseq) separately, in Step 4 Testing, we examined in independent cohorts from the ones used for discovery or validation whether the top candidate biomarkers after the first three steps can assess high pain states, as well as predict future emergency room visits due to pain (Fig. 1 and Table S1), using electronic medical records follow-up data of our study subjects (up to 17.2 years from initial visit at the time of the analyses). Similar to what we did for the validation step, the gene expression data in the test cohorts was normalized (Z-scored) by gender, before those groups were combined. This permits them to be combined. We used as predictors biomarker levels information cross-sectionally, as well as expanded longitudinal information about biomarker levels and slope at multiple visits. We tested the biomarkers in all subjects in the independent test cohort, as well as in a more personalized fashion by gender (Fig. 1).

Convergent functional evidence (CFE)

For the top biomarkers ($n = 56$), we computed into a convergent functional evidence (CFE) score all the evidence from discovery (up to 6 points), CFG prioritization (up to 12 points), validation (up to 6 points), and testing (predicting state high pain, first year ER visits for pain, all future ER visits for pain- up to 4 points each if it significantly predicts in all subjects, 2 points if in gender). The total score can be up to 36 points: 24 from our own new data, and 12 from literature data used for CFG. We weigh our new data more than the literature data, as it is functionally related to pain in 6 independent cohorts (discovery, validation, testing × 2 platforms, microarrays and RNAseq). The goal is to highlight, based on the totality of our data and of the evidence in the field to date, biomarkers that have all around evidence: track pain, have convergent evidence for involvement in pain, as well as predict pain state and future clinical events (Table 1).

The data from the two platforms was compared and integrated at the time of Step 4, as described in Methods.

The top blood biomarkers with the strongest overall convergent functional evidence (CFE) for tracking and predicting pain, after all four steps, in independent cohorts run on two separate platforms (Table 1) were, in descending order of combined CFE4 score: CD55 (CD55 Molecule -Cromer Blood Group), PLAUR (Plasminogen Activator, Urokinase Receptor), RIPOR2 (RHO Family Interacting Cell Polarization Regulator 2), DENND3 (DENN Domain Containing 3), IL1R1 (Interleukin 1 Receptor Type 1), IRAG1 (Inositol 1,4,5-Triphosphate Receptor Associated 1), MAPK1 (Mitogen-Activated Protein Kinase 1), PLCG2 (Phospholipase C Gamma 2), RARA (Retinoic Acid Receptor Alpha), ANXA1 (Annexin A1), CLK4 (CDC Like Kinase 4), CSRNP1 (Cysteine And Serine Rich Nuclear Protein 1), NFE2L2 (NFE2 Like BZIP Transcription Factor 2), RHOA (Ras Homolog Family Member A), CTSB (Cathepsin B), GZMK (Granzyme K), MAEA (Macrophage Erythroblast Attacher, E3 Ubiquitin Ligase), RPL31 (Ribosomal Protein L31), RPS24 (Ribosomal Protein S24), RPSA (Ribosomal Protein SA), TSC22D4 (TSC22 Domain Family Member 4), GLUL (Glutamate-Ammonia Ligase), ILRUN (Inflammation And Lipid Regulator With UBA-Like And NBR1-Like Domains), LILRB3 (Leukocyte Immunoglobulin Like Receptor B3), MIDN (Midnolin), RFX2 (Regulatory Factor X2), SNCA (Synuclein Alpha), WASF2 (WASP Family Member 2), CCNL2 (Cyclin L2), CUX1 (Cut Like Homeobox 1), MBNL3 (Muscleblind Like Splicing Regulator 3), RPL13A (Ribosomal Protein L13a), BANP (BTG3 Associated Nuclear Protein), FUT7 (Fucosyltransferase 7), MAP2K7 (Mitogen-Activated Protein Kinase Kinase 7), RNF10 (Ring Finger Protein 10), CEP57 (Centrosomal Protein 57), GIT2 (GIT ArfGAP 2), MICAL2 (Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2), CD52 (CD52 Molecule), KDM4B (Lysine Demethylase 4B), MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1), NOTCH1 (Notch Receptor 1), RNF123 (Ring Finger Protein 123), SEMA4A (Semaphorin 4A), ELF4 (E74 Like ETS Transcription Factor 4), HES1 (Hes Family BHLH Transcription Factor 1), SLC6A8 (Solute Carrier Family 6 Member 8), YBX3 (Y-Box Binding Protein 3), NFKB1 (Nuclear Factor Kappa B Subunit 1), HLA-B (Major Histocompatibility Complex, Class I, B), MARCHF8 (Membrane Associated Ring-CH-Type Finger 8), MKRN1 (Makorin Ring Finger Protein 1), TRIM58 (Tripartite Motif Containing 58), CRY1 (Cryptochrome Circadian Regulator 1), and APBA3 (Amyloid Beta Precursor Protein Binding Family A Member 3).

CD55 (Complement decay-accelerating factor), the overall top biomarker for pain in this study, plays an essential role in regulating the effect of complement on cell surfaces. Complement activation is involved in cell death and inflammation. CD55 inactivates it, preventing inflammation and cell damage. It is decreased in expression in high pain states in our study, i.e. a "pain -suppressor" gene, which is consistent then with increased cell destruction and inflammation. CD55 has previous, convergent evidence for involvement in pain. It is decreased in expression in dorsal root ganglia in pain in animal model studies [18, 19]. CD55 in our studies modestly predicts high pain state in all patients in the independent testing cohort (AUC 59%, $p = 0.002$). It also modestly predicts future ER visits for pain in future years in all (OR 1.13, $p = 0.007$). CD55 is increased in expression by carbamazepine [20] and dexamethasone [21], which are used to treat pain, but also by lithium [22], valproate [23], sertraline [24], and ginseng [25].

Similarly, ANXA1 (Annexin A1), the top increased in expression biomarker, is a gene that is an effector of glucocorticoid-mediated responses and regulator of the inflammatory processes. It is increased in expression in high pain states in our study, i.e. an "algogene". ANXA1 has previous, convergent evidence for involvement in pain. It is increased in expression in dorsal root ganglia in pain [26], as well as in blood in chronic spinal cord injury pain and in nocioceptive pain [27]. ANXA1 in our studies predicts chronic high pain in all patients in the independent testing cohort (AUC 66%, $p = 0.008$). ANAX1 is decreased in expression by lithium [28] and minocycline [29], as well as by magnesium [30] and omega-3 fatty acids [31].

Biological understanding

Biological pathways. We carried out biological pathway analyses using the list of top biomarkers for pain ($n = 56$ genes). The top pathways were related to neuroinflammation signaling and TNF cellular response (Table 2A). Inflammatory disorders and ankylosing spondylitis were top diseases identified by the pathway analyses programs, pointing out to a molecular underpinning for these well-known clinical co-morbidities (Table 2B).

Networks and interactions. We carried out a STRING analysis (Figure S3) of the top candidate biomarkers that revealed groups of interacting proteins. These networks may have biological significance and could be targeted therapeutically.

Therapeutics

Overall, surprisingly, lithium (42.9%) had the best evidence for broad efficacy in pain (Table 3A), followed by omega-3 fatty acids (28.6%) and ketamine (23.2%). Interestingly, two steroids which can be used in pain, dexamethasone and prednisolone, were lower (14.3%), then carbamazepine, which is used for neuropathic pain, was at 10.7%. Acetaminophen and ibuprofen, standard OTC pain medications, were only at 1.8% each. Omega-3 fatty acids

(28.6%) was the top nutraceutical and may be a widely deployable preventive treatment, with minimal side-effects, including in women who are or may become pregnant.

Best predictive biomarkers

In Step 4, we identified best predictive biomarkers for pain state and trait (first year, and all future ER visits for pain), using cross-sectional and longitudinal methodology (Fig. 2). In an additional Step 5, all the nominally significant biomarkers were re-tested for ability to predict using the whole population used in the study ($n = 1127$), to establish generalizability and avoid an overfit to the testing cohort. The best predictive biomarkers in all, and for each gender, male and female, can be combined in panels to generate reports for doctors, as shown in Fig. 3.

DISCUSSION

We describe novel and comprehensive efforts to advance precision medicine approaches for pain. The top blood biomarkers were discovered, validated and tested in multiple independent cohorts, on two different platforms, to evaluate predictive ability and clinical validity. These biomarkers also open a window into understanding the biology of pain, as well as indicate new and more precise therapeutic approaches.

Current clinical practice and the need for biomarkers

Assessing a persons' internal subjective perceptions and thoughts, along with more objective external ratings of actions and behaviors, are used in clinical practice to assess pain. Such an approach is insufficient, and lagging those used in other medical disorders. Moreover, individuals do not always report accurately their pain, or the clinician does not take them seriously, leading to missed opportunities to intervene and help. Blood biomarkers related to pain, if used as part of pain-driven clinical visits and even routine primary care annual exams, would provide a critical objective measurement to inform clinical assessment, treatment, and ultimately prevention.

Advantages of biomarkers

Blood biomarkers offer real-world clinical practice advantages. As the brain cannot be readily biopsied in live individuals, and CSF is less easily accessible than blood, we have endeavored over the years to identify blood biomarkers for neuropsychiatric disorders, and for pain. A whole –blood approach facilitates field deployment of sample collection. The assessment of gene expression changes focuses our approach on immune cells. The ability to identify peripheral gene expression changes that reflect brain activities is likely due to the fact that the brain and immune system have developmental commonalities, marked by shared reactivity and ensuing gene expression patterns. There is also a bi-directional interaction between the brain and immune system. Not all changes in expression in peripheral cells are reflective of or germane to brain activity. By carefully tracking a phenotype with our within-subject design in the discovery step, and then using convergent functional genomics prioritization, we are able to extract the peripheral changes that do track and are relevant to the brain activity studied, in this case pain. Subsequent validation and testing in independent cohorts narrow the list to the best markers. In the end, we do not expect to recapitulate in the blood all that happens in the brain. We just want to have good accessible peripheral biomarkers- "liquid biopsies", as they are called in cancer.

Comprehensiveness

Our primary goal was to discover and validate biomarkers for pain, that are transdiagnostic. Secondarily, we aimed to understand their universality in all vs. their specificity by gender.

Our studies were arranged in a stepwise fashion. In each of the two studies, Affymetrix and RNAseq, first we endeavored to discover blood gene expression biomarkers for pain using a longitudinal design, looking at differential expression of genes in the blood of male and female subjects with major psychiatric disorders (bipolar disorder, major depressive disorder, schizophrenia/schizoaffective, and post-traumatic stress disorder (PTSD)), high risk populations prone to pain, which constitute and enriched pool in which to look for biomarkers. We compared low pain states to high pain states using a powerful within-subject design [8, 9, 11, 32], to generate a list of differentially expressed genes. Second, we used a comprehensive Convergent Functional Genomics (CFG) approach with the whole body of knowledge in the field to prioritize from the list of differentially expressed genes/biomarkers of relevance to pain. CFG integrates multiple independent lines of evidence- genetic, gene expression, and protein data, from brain and periphery, from human studies, as a Bayesian strategy for identifying and prioritizing findings, reducing the false-positives and false-negatives inherent in each individual approach. Third, we examined if the expression levels of the top biomarkers identified by us as tracking pain state are changed even more strongly in blood samples from an independent cohort of subjects who had chronic pain, to validate these biomarkers. Fourth, the top candidate biomarkers thus discovered, prioritized, and validated that converged between the two studies, were tested for predictive ability in corresponding independent cohorts of psychiatric subjects. Fifth, they were tested for overall generalizability in the whole mothership of samples from the two studies. Lastly we exemplified the use of panels of biomarkers in reports for physicians, to predict state and one-year risk, and to match patients to medications and nutraceuticals. We also demonstrate the bio-socio-psychological integration of our biomarker scores with clinical rating scales and subjective self-report (Fig. 3). This series of studies was a systematic and comprehensive approach to move the field forward towards precision medicine.

Power

We used a systematic discovery, prioritization, validation, and testing approach, as we have done over the years for pain and for other disorders [3, 10, 12, 13, 33, 34]. For discovery, we used a hard to accomplish but powerful within-subject design. A within-subject design factors out genetic variability, as well as some medications, lifestyle, and demographic effects on gene expression, permitting identification of relevant signal with Ns as small as 1 [32]. Another benefit of a within-subject design may be accuracy/consistency of psychiatric symptoms ("phene expression"), as it is the same person reporting different states. This is similar in rationale to the signal detection benefits it provides in gene expression.

Based on our work of over two decades in genetics and gene expression, along with the results of others in the field, we estimate that using a quantitative phenotype is up to 1 order of magnitude more powerful than using a categorical diagnosis. The within-subject longitudinal design, by factoring out all genetic and some environmental variability, is up to 3 orders of magnitude more powerful than an inter-subject case-control cross-sectional design. Moreover, gene expression, by integrating the effects of many SNPs and environment, is up to 3 orders of magnitude more powerful than a genetic study. Combined, our approach may be up to 6 orders of magnitude more powerful than a GWAS study, even prior to the CFG literature-based prioritization step, which encompasses all the independent work in the field prior to our studies, which may add up to 1 order of magnitude as well. In addition, the Validation and the Testing steps add additional 1 order of magnitude power each. As such, our approach might be up to 10 orders of magnitude more powered to detect signal than most current genetic study designs as used in GWAS.

Reproducibility

We reproduced and expanded our earlier biomarker findings [10]. 66% of our top 56 biomarkers from the current work were

identified in the Discovery step our 2019 study, that was smaller and was done on a single platform, Affymetrix.

Additionally, there is reproducibility of our candidate biomarkers from Discovery with findings generated by other independent studies as part of the Step 2 Prioritization using Convergent Functional Genomics (see Table S2). This independent reproducibility of findings between our studies and these other studies, which are done in independent cohorts from ours, with independent methodologies, is reassuring, and provides strong convergent evidence for the validity and relevance of our approach and of their approaches. Our work also provides functional evidence for some of their top genetic hits.

Lastly, after our study was completed, another study came out that independently identified two of our top biomarkers, RHOA and ILRUN, as genes involved in low-back pain [35].

Pathophysiology

Top biological pathways have to do with neuroinflammation signaling, and cellular response to TNF (Table 2A). Pain may be a whole-body over-reaction in response to an adverse environment. TNF contributes to pain by enhancing inflammation, sensitizing pain pathways, and modulating neural responses, peripherally and centrally. Its role underscores the complex interaction between the immune system and pain perception. However, treatments targeting TNF must be approached cautiously due to the cytokine's broader roles in immune regulation and potential side effects.

The majority of top blood biomarkers we have identified have prior evidence in human data from pain, which indicates their relevance to the pathophysiology of pain (Table S2). The co-directionality of blood changes in our work and brain changes reported in the literature needs to be interpreted with caution, as it may depend on tissue, etc.

The top candidate biomarkers also had prior evidence of involvement in other psychiatric and related disorders (Table 1 and S3, Fig. 1C), providing a molecular basis for co-morbidity, and the possible predisposing effects of some these disorders on pain. In particular, direct comparisons with our previous blood biomarkers studies for other disorders (Fig. 1C) revealed the most overlap with hallucinations by far. This suggests that some degree of sensory excess and connection abnormality occurs, providing a possible basis for nocioplastic pain (persistent pain after the initial lesion has resolved). There was also a significant degree of molecular co-morbidity with aging (Table S6), consistent with the idea that chronic pain may not only reduce health span but also lifespan.

Phenomenology

We have also looked at subtypes of pain in the subjects from the discovery cohort while they were in a high pain state. We identified 16 subtypes, based on two-way unsupervised hierarchical clustering on measures of stress, anxiety, mood and psychosis (Figure S3). The subtypes with the most subsequent ER visits in the year following testing tended to have high depression as a common factor.

Biomarkers vs. scales

In general, the best predictive biomarkers were better than the rating scales at predicting future ER visits in females, but not in males (Table S5). This may reflect the fact that these are difficult phenotypes to assess by clinicians, driven by psycho-social factors also (see Fig. 3), and reinforces the need for using objective blood biomarkers to assess pain.

Diagnostics

For the biomarkers identified by us, combining all the available evidence from this current work into a convergent functional evidence (CFE) score, brings to the fore biomarkers that have clinical validity for objective assessment and risk prediction for pain (Table 1). These biomarkers should be tested individually as well as tested as polygenic panels of biomarkers in future clinical studies and practical clinical applications in the field, as we show in Fig. 3. They may permit to distinguish, upon an initial clinical presentation of pain, whether the person is in fact severely in pain and at chronic risk (Fig. 2). The integration of phenomic data, such as repeated measures of VAS Pain (perhaps via a phone app in a daily fashion), can further substantiate and elucidate pain risk, distinguishing between an intermittent type such as transient pain, and continuous type such as chronic pain.

Predictions for pain were in general stronger in women than in men (Table 1 and Fig. 2), by an order of 10–20% points on AUCs. While some of it may be biological, in terms of immune system reactivity and brain-blood interplay being perhaps higher in women, it is also possible that men are not as accurate as women in terms of reporting pain symptoms (affecting our results on state predictions), and may seek controlled substances more (affecting our results on future ER visits predictions). If so, this misreporting makes the use of objective biomarker tests in men even more necessary.

In regard to how our biomarker discoveries might be applied in clinical laboratory settings, we suggest that panels of top biomarkers for pain be used (Fig. 3). In practice, every new patient tested would be normalized against the database of similar patients already tested, and compared to them for ranking and risk prediction purposes, regardless if a platform like microarrays, RNA sequencing, or a more targeted one like PCR is used in the end clinically. As databases get larger, normative population levels can and should be established, similar to any other laboratory measures. Moreover, longitudinal monitoring of changes in biomarkers within an individual, measuring most recent slope of change, maximum levels attained, and maximum slope of change attained in the past, may be even more informative than simple cross-sectional comparisons of levels within an individual with normative populational levels, as we have shown in our studies. For future point of care approaches, research and development should focus on top individual biomarkers, including at a protein level. One might look at a combination of the best universal biomarkers (that are predictive in all), for reliability, and of the best personalized biomarkers (that are predictive by gender, and even diagnosis), for higher accuracy.

Treatment

Biomarkers may also be useful for matching patients to medications and measuring response to treatment (pharmacogenomics) (Fig. 3, Table 3A and S4), as well as new drug discovery clinical trials, and drug repositioning (Table 3B). From the pharmacogenomics analyses, lithium was a top hit, second were omega-3 fatty acids. Other interesting matches were ketamine, magnesium, vortioxetine. All these drugs and nutraceuticals are relatively safe if used appropriately, and have been used in clinical practice for other indications for decades, which facilitates the direct translation to clinical practice of our findings. The fact that medications currently used for the treatment of pain were lower on the list is striking, and suggests there is room for improvement.

Drug repurposing analyses identified betablockers such as carvedilol, immune suppressants such as sirolimus (rapamycin), steroids such as budesonide, and versatile antipsychotics such as quetiapine, as potential choices, as well as nutraceuticals such as berbamine, inositol, naringein, and curcumin (Table 3B).

CONCLUSIONS

In this current work, we carried out extensive blood gene expression studies in male and female subjects with major psychiatric disorders, an enriched population in terms of co-morbidity with pain- neuropathic, nocioceptive, and nocioplastic.

The potential molecular-level co-morbidity between psychiatric disorders and pain is underlined by the fact that medications for anxiety and mood disorders (anticonvulsants, antidepressants) are also used to treat pain. The novel potential utility of lithium was highlighted by our studies. Our work also uncovered a molecular overlap with hallucinations (Fig. 1C), and the potential utility of quetiapine, and antipsychotic. Second was an overlap with stress, and the potential utility of a beta-blocker, carvedilol. These last two findings may be relevant to nocioplastic pain, a particularly pernicious and refractory type of pain.

Overall, this work is a major step forward towards better understanding, diagnosing, and treating pain. Taken together, our data supports the possibility that biologically, pain is a disorder of over-reactivity, acute and in some cases persistent, even after the initial physical trauma has gone. This is analogous to, and in fact overlapping with, psychological trauma and PTSD. Stress needs to be actively addressed and mitigated in high-risk individuals and circumstances, in both men [36], and women [37]. We hope that our trait biomarkers for future risk may be useful in preventive approaches, before full-blown chronic pain manifests itself (or re-occurs). The two cases of subjects who completed out testing and we generated reports on illustrates the power of our approach to identify risk (Fig. 3). Prevention could be accomplished with biological interventions (i.e., early targeted use of medications or nutraceuticals), physical pre-habilitation, and psychological support for stress. Given the fact that pain disorders are on the increase in the US and worldwide, that pain can severely affect quality of life and lead to shortened lifespans, that not all patients respond to current treatments, and that some of the current treatments, particularly opioids, are addictive, the need for and importance of efforts such as ours cannot be overstated.

DATA AVAILABILITY

The data that support the findings of this study are not openly available due to reasons of privacy and sensitivity. They are available from the corresponding author upon reasonable request. Please send correspondence to A.B. Niculescu (aniculescu@arizona.edu).

REFERENCES

1. Dahlhamer J, Lucas J, Zelaya C, Nahin R, Mackey S, DeBar L, et al. Prevalence of chronic pain and high-impact chronic pain among adults - United States, 2016. MMWR Morb Mortal Wkly Rep. 2018;67:1001–6.
2. Hooten WM. Chronic pain and mental health disorders: shared neural mechanisms, epidemiology, and treatment. Mayo Clin Proc. 2016;91:955–70.
3. Niculescu AB, Le-Niculescu H, Levey DF, Roseberry K, Soe KC, Rogers J, et al. Towards precision medicine for pain: diagnostic biomarkers and repurposed drugs. Mol Psychiatry. 2019;24:501–22.
4. Fundaun J, Kolski M, Molina-Alvarez M, Baskozos G, Schmid AB. Types and concentrations of blood-based biomarkers in adults with peripheral neuropathies: a systematic review and meta-analysis. JAMA Netw Open. 2022;5:e2248593.
5. Kashanian A, Tsolaki E, Caruso J, Bari A, Pouratian N. Imaging as a pain biomarker. Neurosurg Clin N Am. 2022;33:345–50.
6. Johnston KJA, Adams MJ, Nicholl BI, Ward J, Strawbridge RJ, Ferguson A, et al. Genome-wide association study of multisite chronic pain in UK biobank. PLoS Genet. 2019;15:e1008164.
7. Mercer Lindsay N, Chen C, Gilam G, Mackey S, Scherrer G. Brain circuits for pain and its treatment. Sci Transl Med. 2021;13:eabj7360.
8. Le-Niculescu H, Levey DF, Ayalew M, Palmer L, Gavrin LM, Jain N, et al. Discovery and validation of blood biomarkers for suicidality. Mol Psychiatry. 2013;18:1249–64.
9. Niculescu AB, Levey DF, Phalen PL, Le-Niculescu H, Dainton HD, Jain N, et al. Understanding and predicting suicidality using a combined genomic and clinical risk assessment approach. Mol Psychiatry. 2015;20:1266–85.
10. Niculescu AB, Le-Niculescu H, Levey DF, Phalen PL, Dainton HL, Roseberry K, et al. Precision medicine for suicidality: from universality to subtypes and personalization. Mol Psychiatry. 2017;22:1250–73.
11. Levey DF, Niculescu EM, Le-Niculescu H, Dainton HL, Phalen PL, Ladd TB, et al. Towards understanding and predicting suicidality in women: biomarkers and clinical risk assessment. Mol Psychiatry. 2016;21:768–85.
12. Le-Niculescu H, Roseberry K, Gill SS, Levey DF, Phalen PL, Mullen J, et al. Precision medicine for mood disorders: objective assessment, risk prediction, pharmacogenomics, and repurposed drugs. Mol Psychiatry. 2021;26:2776–804.
13. Roseberry K, Le-Niculescu H, Levey DF, Bhagar R, Soe K, Rogers J, et al. Towards precision medicine for anxiety disorders: objective assessment, risk prediction, pharmacogenomics, and repurposed drugs. Mol Psychiatry. 2023;28:2894–912.
14. Hill MD, Gill SS, Le-Niculescu H, MacKie O, Bhagar R, Roseberry K, et al. Precision medicine for psychotic disorders: objective assessment, risk prediction, and pharmacogenomics. Mol Psychiatry. 2024;29:1528–49.
15. Bhagar R, Gill SS, Le-Niculescu H, Yin C, Roseberry K, Mullen J, et al. Next-generation precision medicine for suicidality prevention. Transl Psychiatry. 2024;14:362.
16. Ramos A, Ishizuka K, Hayashida A, Namkung H, Hayes LN, Srivastava R, et al. Nuclear GAPDH in cortical microglia mediates cellular stress-induced cognitive inflexibility. Mol Psychiatry. 2024;29:2967–78.
17. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell. 2017;171:1437–52.e1417
18. Stephens KE, Zhou W, Renfro Z, Ji Z, Ji H, Guan Y, et al. Global gene expression and chromatin accessibility of the peripheral nervous system in animal models of persistent pain. J Neuroinflammation. 2021;18:185.
19. LaCroix-Fralish ML, Austin JS, Zheng FY, Levitin DJ, Mogil JS. Patterns of pain: meta-analysis of microarray studies of pain. Pain. 2011;152:1888–98.
20. Schulpen SH, Pennings JL, Piersma AH. Gene expression regulation and pathway analysis after valproic acid and carbamazepine exposure in a human embryonic stem cell-based neurodevelopmental toxicity assay. Toxicol Sci. 2015;146:311–20.
21. Menke A, Arloth J, Putz B, Weber P, Klengel T, Mehta D, et al. Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. Neuropsychopharmacology. 2012;37:1455–64.
22. Cattane N, Courtin C, Mombelli E, Maj C, Mora C, Etain B, et al. Transcriptomics and miRNomics data integration in lymphoblastoid cells highlights the key role of immune-related functions in lithium treatment response in bipolar disorder. BMC Psychiatry. 2022;22:665.
23. Hill EJ, Nagel DA, O'Neil JD, Torr E, Woehrling EK, Devitt A, et al. Effects of lithium and valproic acid on gene expression and phenotypic markers in an NT2 neurosphere model of neural development. PLoS ONE. 2013;8:e58822.
24. Funayama Y, Li H, Ishimori E, Kawatake-Kuno A, Inaba H, Yamagata H, et al. Antidepressant response and stress resilience are promoted by CART peptides in GABAergic neurons of the anterior cingulate cortex. Biol Psychiatry Glob Open Sci. 2023;3:87–98.
25. Yan J, Ma Y, Zhao F, Gu W, Jiao Y. Identification of immunomodulatory signatures induced by american ginseng in murine immune cells. Evid Based Complement Alternat Med. 2013;2013:972814.
26. Tavares-Ferreira D, Shiers S, Ray PR, Wangzhou A, Jeevakumar V, Sankaranarayanan I, et al. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci Transl Med. 2022;14:eabj8186.
27. Morrison D, Arcese AA, Parrish J, Gibbs K, Beaufort A, Herman P, et al. Systemic gene expression profiles according to pain types in individuals with chronic spinal cord injury. Mol Pain. 2021;17:17448069211007289.
28. Kittel-Schneider S, Hilscher M, Scholz CJ, Weber H, Grunewald L, Schwarz R, et al. Lithium-induced gene expression alterations in two peripheral cell models of bipolar disorder. World J Biol Psychiatry. 2019;20:462–75.
29. He L, Xu R, Chen Y, Liu X, Pan Y, Cao S, et al. Intra-CA1 administration of minocycline alters the expression of inflammation-related genes in hippocampus of CCI rats. Front Mol Neurosci. 2019;12:248.
30. Almousa LA, Salter AM, Castellanos M, May ST, Langley-Evans SC. The response of the human umbilical vein endothelial cell transcriptome to variation in magnesium concentration. Nutrients. 2022;14:3586.
31. Le-Niculescu H, Case NJ, Hulvershorn L, Patel SD, Bowker D, Gupta J, et al. Convergent functional genomic studies of omega-3 fatty acids in stress reactivity, bipolar disorder and alcoholism. Transl Psychiatry. 2011;1:e4.
32. Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Miriami E, et al. Personal omics profiling reveals dynamic molecular and medical phenotypes. Cell. 2012;148:1293–307.
33. Le-Niculescu H, Roseberry K, Levey DF, Rogers J, Kosary K, Prabha S, et al. Towards precision medicine for stress disorders: diagnostic biomarkers and targeted drugs. Mol Psychiatry. 2020;25:918–38.
34. Niculescu AB, Le-Niculescu H, Roseberry K, Wang S, Hart J, Kaur A, et al. Blood biomarkers for memory: toward early detection of risk for Alzheimer disease, pharmacogenomics, and repurposed drugs. Mol Psychiatry. 2020;25:1651–72.
35. Belonogova NM, Elgaeva EE, Zorkoltseva IV, Kirichenko AV, Svishcheva GR, Freidin MB, et al. A multi-trait approach identified 7 novel genes for back pain. Pain Rep. 2025;10:e1218.

36. Khan AJ, Campbell-Sills L, Sun X, Kessler RC, Adler AB, Jain S, et al. Association between responsibility for the death of others and postdeployment mental health and functioning in US soldiers. JAMA Netw Open. 2021;4:e2130810.
37. Niwa M, Lockhart S, Wood DJ, Yang K, Francis-Oliveira J, Kin K, et al. Prolonged HPA axis dysregulation in postpartum depression associated with adverse early life experiences: a cross-species translational study. Nat Ment Health. 2024;2:593–604.

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AUTHOR CONTRIBUTIONS

ABN designed the study and wrote the manuscript. RB, HLN, SC, AG and CW analyzed the data. JM and MS contributed to bioinformatic pipeline development. MS, AE, and EM assisted with predictions and with sample report generation. AS and FW assisted with data interpretation. SMK oversaw microarray and RNA sequencing experiments. All authors discussed the results and commented on the manuscript.

COMPETING INTERESTS

ABN is listed as inventor on patent applications. ABN and AS are co-founders, SMK is a consultant, MS and AE full-time employees, and EM a part-time employee, of MindX Sciences.

ADDITIONAL INFORMATION

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