EP3765630A2 PRECISION MEDICINE FOR PAIN: DIAGNOSTIC BIOMARKERS, PHARMACOGENOMICS, AND

Available in

Bibliographic data | Description | Claims | Drawings | Original document | Citations | Legal events | Patent family

Data originating from sources other than the EPO may not be accurate, complete, or up to date.

Event indicator	Category	Event description	Countries	Event date	Effective date	Details
EP STAA	W: Other	INFORMATION ON THE STATUS OF AN EP PATENT APPLICATION OR GRANTED EP PATENT		2019-09-21		**Further information** STATUS: THE INTERNATIONAL PUBLICATION HAS BEEN MADE
EP PUAI	Q: Document publication	PUBLIC REFERENCE MADE UNDER ARTICLE 153(3) EPC TO A PUBLISHED INTERNATIONAL APPLICATION THAT HAS ENTERED THE EUROPEAN PHASE		2020-12-18		**Further information** ORIGINAL CODE: 0009012
EP STAA	W: Other	INFORMATION ON THE STATUS OF AN EP PATENT APPLICATION OR GRANTED EP PATENT		2020-12-18		**Further information** STATUS: REQUEST FOR EXAMINATION WAS MADE

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2019 (19.09.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/178379 A2

(51) International Patent Classification:
G01N 33/50 (2006.01)

(21) International Application Number:
PCT/US2019/022305

(22) International Filing Date:
14 March 2019 (14.03.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/642,789 14 March 2018 (14.03.2018) US

(71) Applicants: **INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION** [US/US]; 518 Indiana Ave., Indianapolis, IN 46202 (US). **UNITED STATES GOVERNMENT as represented by THE DEPARTMENT OF VETERANS AFFAIRS** [US/US]; 810 Vermont Avenue N.W., Washington, DC 20420 (US).

(72) Inventor: **NICULESCU, Alexander Bogden**; c/o Indiana University and Research Technology Corporation, 518 Indiana Ave., Indianapolis, IN 46202 (US).

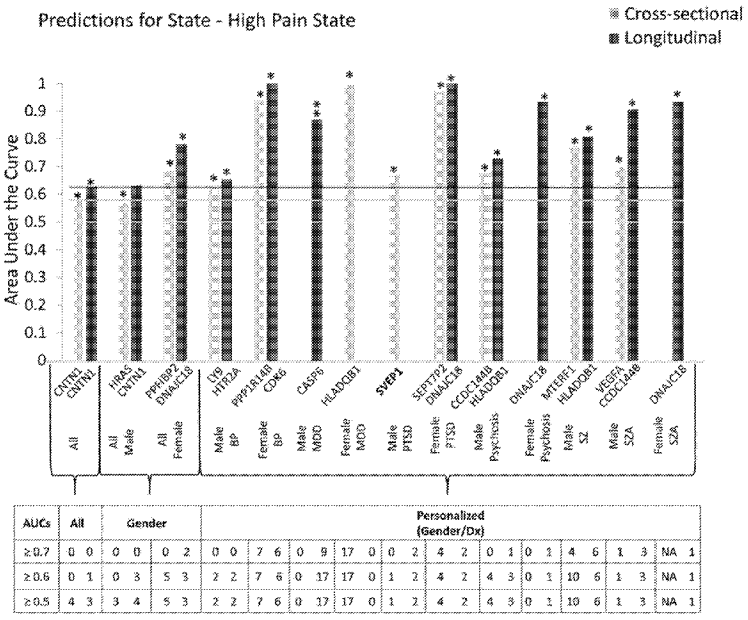
(74) Agent: **BOETTLER, Jeannie, M.** et al.; Stinson Leonard Street LLP, 7700 Forsyth Boulevard, Suite 1100, St. Louis, MO 63105-1821 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: PRECISION MEDICINE FOR PAIN: DIAGNOSTIC BIOMARKERS, PHARMACOGENOMICS, AND REPURPOSED DRUGS

FIG. 2A



AUCs	All		Gender			Personalized (Gender/Dx)																
≥0.7	0	0	0	0	0	2	0	0	7	6	0	9	17	0	0	2	4	2	0	1	0	1	4	6	1	3	NA	1
≥0.6	0	1	0	3	5	3	2	2	7	6	0	17	17	0	1	2	4	2	4	3	0	1	10	6	1	3	NA	1
≥0.5	4	3	3	4	5	3	2	2	7	6	0	17	17	0	1	2	4	2	4	3	0	1	10	6	1	3	NA	1

(57) Abstract: Disclosed are methods for treating pain and tracking response to treatment. Also disclosed are methods for determining pain, including predicting future medical care facility visits for pain.

[Continued on next page]

WO 2019/178379 A2

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

PRECISION MEDICINE FOR PAIN: DIAGNOSTIC BIOMARKERS, PHARMACOGENOMICS, AND REPURPOSED DRUGS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/642,789, filed March 14, 2018, which is hereby incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under OD007363 awarded by the National Institutes of Health and CX000139 merit award by the Veterans Administration. The government has certain rights in the invention.

BACKGROUND OF THE DISCLOSURE

[0003] The present disclosure relates generally to methods for objectively determining and predicting pain. More particularly, the present disclosure relates to methods for tracking pain intensity, predicting levels of pain and predicting future medical facility visits for pain. Also disclosed are drugs and natural compounds identified as candidates for treating pain using biomarker gene expression signatures.

[0004] Pain is a subjective sensation that reflects bodily damage and the possibility of future harm. Pain treatment is a multi-billion dollar market in the United States. The United States is, however, experiencing an opioid abuse epidemic.

[0005] Mental states can affect the perception of pain, and in turn, can be affected by pain. Psychiatric patients may have an increased perception of pain, as well as increased physical health reasons for pain due to their often adverse life trajectory.

[0006] Currently, there are no objective tests for determining pain, so clinicians must rely on self-reporting by patients. An objective test for pain can facilitate proper diagnosis and treatment, enabling more confident treatment for those needing treatment for pain, and avoid over-prescribing of potentially addictive medications to those not in need. Blood biomarkers for pain can serve as companion diagnostics for clinical trials for the development of new pain medications and repurposing existing drugs for use as pain treatments. Accordingly, there exists a need for objective measures for determining pain, which can guide appropriate treatment.

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure relates generally to methods for determining and predicting pain. More particularly, the present disclosure relates to methods for objectively determining pain intensity, predicting future emergency department (ED) visits for pain. Also disclosed are methods for identifying drug and natural compounds as candidates for treating pain using biomarker gene expression signatures.

[0008] In one aspect, the present disclosure is directed to a method for determining pain intensity in a subject in need thereof. The method comprises: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of a blood biomarker; and identifying a difference between the expression level of the blood biomarker in a sample obtained from the subject and the reference expression level of a blood biomarker, wherein the difference in the expression level of the blood biomarker in the sample obtained from the subject and the reference expression level of the blood biomarker determines pain intensity. In one embodiment, the blood biomarker is a panel of blood biomarkers. The reference level can be an average or reference range in the population (a "cross-sectional" approach), or it can be the level of a sample obtained previously in the subject when the subject was not in need of treating pain (a "longitudinal" approach).

[0009] In another aspect, the present disclosure is directed to a method for identifying a blood biomarker for pain, the method comprising: obtaining a first biological sample from a subject and administering a first pain intensity test to the subject; obtaining a second biological sample from the subject and administering a second pain intensity test to the subject; identifying a first cohort of subjects by identifying subjects having a change from low pain intensity to high pain intensity as determined by a difference between the first pain intensity test and the second pain intensity test; identifying candidate biomarkers in the first cohort by identifying biomarkers having a change in expression between the first biological sample and the second biological sample.

[0010] In one aspect, the present disclosure is directed to a method for predicting future emergency department (ED) visits for pain. The method comprises: obtaining an expression level of a blood biomarker or panel of blood biomarkers in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker or panel of blood biomarkers; identifying a difference in the expression level of the blood biomarkers in the sample and the

reference expression level of the blood biomarkers; wherein the difference in the expression level of the blood biomarkers in the sample obtained from the subject and the reference expression level of the blood biomarkers determines the likelihood of future ED visits for pain. In one embodiment, the blood biomarker is a panel of blood biomarkers. The reference expression level can be that as described herein.

[0011] In another aspect, the present disclosure is directed to a method for mitigating pain in a subject in need thereof. The method comprises: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker; and administering a treatment, wherein the treatment reduces the difference between the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker to mitigate pain in the subject. In one embodiment, the blood biomarker is a panel of blood biomarkers. The reference expression level can be that as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The disclosure will be better understood, and features, aspects and advantages other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such detailed description makes reference to the following drawings, wherein:

[0013] FIGS. 1A-1G depict Steps 1-3: Discovery, Prioritization and Validation. FIG. 1A depicts Cohorts used in study, depicting flow of discovery, prioritization, and validation of biomarkers from each step. FIG. 1B depicts Discovery cohort longitudinal within-participant analysis. Phchp### is study ID for each participant. V# denotes visit number. FIG. 1C depicts Discovery of possible subtypes of Pain based on High Pain visits in the discovery cohort. Participants were clustered using measures of mood and anxiety (Simplified Affective State Scale (SASS)), as well as psychosis (PANNS Positive) FIG. 1D depicts Differential gene expression in the Discovery cohort -number of genes identified with differential expression (DE) and absent-present (AP) methods with an internal score of 1 and above. Red/Underlined-increased in expression in High Pain, blue/Bold-decreased in expression in High Pain. At the discovery step probesets are identified based on their score for tracking pain with a maximum of internal points of 6 (33% (2pt), 50% (4pt) and 80% (6pt)). FIG. 1E depicts prioritization with

CFG for prior evidence of involvement in pain. In the prioritization step probesets are converted to their associated genes using Affymetrix annotation and GeneCards. Genes are prioritized and scored using CFG for pain evidence with a maximum of 12 external points. Genes scoring at least 6 points out of a maximum possible of 18 total internal and external scores points are carried to the validation step. FIG. 1F depicts Validation in an independent cohort of psychiatric patients with co-morbid pain disorders and severe subjective and functional pain ratings. In the validation step biomarkers are assessed for stepwise change from the discovery groups of participants with Low Pain, to High Pain, to Clinically Severe Pain disorder, using ANOVA. N= number of testing visits. 5 biomarkers were nominally significant, MFAP3 and PIK3CD were the most significant, and 68 biomarkers were stepwise changed.

[0014] FIGS. 2A-2C depict Best Single Biomarkers Predictors for State Predictions (FIG. 2A), Trait Predictions First Year (FIG. 2B), and Trait Predictions All Future Years (FIG. 2C). From the long list (n=65). Those on short list (n= 5) are bolded. Bar graph shows best predictive biomarkers in each group. * Nominally significant $p<0.05$. ** Bonferroni significant for the 65 biomarkers tested. Table underneath the figures displays the actual number of biomarkers for each group whose ROC AUC p-values were at least nominally significant. Some female diagnostic groups were omitted from the graph as they did not have any significant biomarkers. Cross-sectional was based on levels at one visit. Longitudinal was based on levels at multiple visits (integrates levels at most recent visit, maximum levels, slope into most recent visit, and maximum slope). Dividing lines represent the cutoffs for a test performing at chance levels (white), and at the same level as the best biomarkers for all subjects in cross-sectional (gray) and longitudinal (black) based predictions. All biomarkers performed better than chance. Biomarkers also performed better when personalized by gender and diagnosis.

[0015] FIG. 3 depicts the pain scale of male and female psychiatric participants.

[0016] FIG. 4 depicts the STRING interaction network for 60 top biomarkers for pain.

[0017] While the disclosure is susceptible to various modifications and alternative forms, specific embodiments thereof have been shown by way of example in the drawings and are herein described below in detail. It should be understood, however, that the description of specific embodiments is not intended to limit the disclosure to cover all modifications, equivalents and alternatives falling within the spirit and scope of the disclosure as defined by the appended claims.

DETAILED DESCRIPTION

[0018] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar to or equivalent to those described herein may be used in the practice or testing of the present disclosure, the preferred materials and methods are described below.

[0019] In accordance with the present disclosure, methods have been developed to objectively determine pain intensity and predict future emergency department (ED) visits for pain.

[0020] In some embodiments, the methods of the present disclosure as described herein are intended to include the use of such methods in "at risk" subjects, including subjects unaffected by or not otherwise afflicted with pain as described herein, for the purpose of diagnosing, prognosing and identifying subjects such that treatment, treatment planning, and treatment options for pain can be made. As used herein, a subject "at risk for pain" refers to individuals who may develop pain. As such, in some embodiments, the methods disclosed herein are directed to a subset of the general population such that, in these embodiments, not all of the general population may benefit from the methods. Based on the foregoing, because some of the method embodiments of the present disclosure are directed to specific subsets or subclasses of identified subjects (that is, the subset or subclass of subjects "at risk for" the specific conditions noted herein), not all subjects will fall within the subset or subclass of subjects as described herein.

[0021] Particularly suitable subjects are humans. Suitable subjects can also be experimental animals such as, for example, monkeys and rodents, that display a behavioral phenotype associated with pain. In one particular aspect, the subject is a female human. In another particular aspect, the subject is a male human.

[0022] Suitable samples can be, for example, saliva, blood, plasma, serum and a cheek swab. The samples can be further processed using methods known to those skilled in the art to isolate molecules contained in the sample such as, for example, cells, proteins and nucleic acids (e.g., DNA and RNA).

[0023] The isolated molecules can also be further processed. For example, cells can be lysed and subjected to methods for isolating proteins and/or nucleic acids contained within the cells. Proteins and nucleic acids contained in the sample and/or in isolated cells can be processed. For example, proteins can be processed for electrophoresis, Western blot analysis, immunoprecipitation and combinations thereof. Nucleic acids can be processed, for example, for polymerase chain reaction, electrophoresis, Northern blot analysis, Southern blot analysis, RNase protection assays, microarrays, serial analysis of gene expression (SAGE) and combinations thereof.

[0024] Suitable probes are described herein and can include, for example, nucleic acid probes, antibody probes, and chemical probes.

[0025] In some embodiments, the probe can be a labeled probe. Suitable labels can be, for example, a fluorescent label, an enzyme label, a radioactive label, a chemical label, and combinations thereof. Suitable radioactive labels are known to those skilled in the art and can be a radioisotope such as, for example, ^{32}P, ^{33}P, ^{35}S, ^{3}H and ^{125}I. Suitable enzyme labels can be, for example, colorimetric labels and chemiluminescence labels. Suitable colorimetric (chromogenic) labels can be, for example, alkaline phosphatase, horse radish peroxidase, biotin and digoxigenin. Biotin can be detected using, for example, an anti-biotin antibody, or by streptavidin or avidin or a derivative thereof which retains biotin binding activity conjugated to a chromogenic enzyme such as, for example, alkaline phosphatase and horse radish peroxidase. Digoxigenin can be detected using, for example, an anti-digoxigenin antibody conjugated to a chromogenic enzyme such as, for example, alkaline phosphatase and horse radish peroxidase. Chemiluminescence labels can be, for example, alkaline phosphatase, glucose-6-phosphate dehydrogenase, horseradish peroxidase, Renilla luciferase, and xanthine oxidase. A particularly suitable label can be, for example, SYBR® Green (commercially available from Life Technologies). A particularly suitable probe can be, for example, an oligonucleotide labelled with SYBR® Green. Suitable chemical labels can be, for example, periodate and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC).

[0026] As used herein, “diagnosing” and “diagnosis” are used according to their ordinary meaning as understood by those skilled in the art to refer to determining objectively that a subject has increased pain intensity.

[0027] As used herein, “predicting pain in a subject in need thereof” refers to indicating in advance that a subject is likely to develop or is at risk for developing pain and/or identifying that a subject with pain wherein the pain is likely to increase and/or identifying a subject that will visit a hospital or other medical facility because of pain and/or because of increasing pain.

[0028] As used herein, the term “biomarker” refers to a molecule to be used for analyzing a subject's test sample. Examples of such biomarkers can be nucleic acids (such as, for example, a gene, DNA and RNA), proteins and polypeptides. In particularly preferred embodiments, the biomarker can be the levels of expression of a biomarker gene. Particularly suitable biomarker genes can be, for example, those listed in Tables 1, 4, 5, 7 and combinations thereof.

[0029] As used herein, “a reference expression level of a biomarker” refers to the expression level of a biomarker established for a subject with no pain, expression level of a biomarker in a normal/healthy subject with no pain as determined by one skilled in the art using established methods as described herein, and/or a known expression level of a biomarker obtained from literature. In one suitable embodiment, the reference level can be an average or reference range in the population (a "cross-sectional" approach). In another embodiment, the reference expression level can be the level of a sample obtained previously in the subject when the subject was not in need of treating pain (a "longitudinal" approach). The reference expression level of the biomarker can further refer to the expression level of the biomarker established for a High Pain subject, including a population of High Pain subjects. The reference expression level of the biomarker can also refer to the expression level of the biomarker established for a Low Pain subject, including a population of Low Pain subjects. The reference expression level of the biomarker can also refer to the expression level of the biomarker established for any combination of subjects such as a subject with no pain, expression level of the biomarker in a normal/healthy subject with no pain, expression level of the biomarker for a subject who has pain at the time the sample is obtained from the subject, but who later exhibits increase in pain, expression level of the biomarker as established for a High Pain subject, including a population of High Pain subjects, and expression level of the biomarker can also refer to the expression level of the biomarker established for a Low Pain subject, including a population of Low Pain subjects. The reference expression level of the biomarker can also refer to the expression level of the biomarker obtained from the subject to which the method is applied. As such, the change within a subject from visit to visit can indicate increased or decreased pain. For example, a plurality of

expression levels of a biomarker can be obtained from a plurality of samples obtained from the same subject and used to identify differences between the plurality of expression levels in each sample. Thus, in some embodiments, two or more samples obtained from the same subject can provide an expression level(s) of a blood biomarker and a reference expression level(s) of the blood biomarker.

[0030] As used herein, "expression level of a biomarker" refers to the process by which a gene product is synthesized from a gene encoding the biomarker as known by those skilled in the art. The gene product can be, for example, RNA (ribonucleic acid) and protein. Expression level can be quantitatively measured by methods known by those skilled in the art such as, for example, northern blotting, amplification, polymerase chain reaction, microarray analysis, tag-based technologies (e.g., serial analysis of gene expression and next generation sequencing such as whole transcriptome shotgun sequencing or RNA-Seq), Western blotting, enzyme linked immunosorbent assay (ELISA), and combinations thereof.

[0031] As used herein, a "difference" and/or "change" in the expression level of the biomarker refers to an increase or a decrease in the measured expression level of a blood biomarker when analyzed against a reference expression level of the biomarker. In some embodiments, the "difference" and/or "change" refers to an increase or a decrease by about 1.2-fold or greater in the expression level of the biomarker as identified between a sample obtained from the subject and the reference expression level of the biomarker. In one embodiment, the difference and/or change in expression level is an increase or decrease by about 1.2 fold. As used herein "a risk for pain" can refer to an increased (greater) risk that a subject will experience (or develop) pain. For example, depending on the biomarker(s) selected, the difference and/or change in the expression level of the biomarker(s) can indicate an increased (greater) risk that a subject will experience (or develop) pain. Conversely, depending on the biomarker(s) selected, the difference and/or change in the expression level of the biomarker(s) can indicate a decreased (lower) risk that a subject will experience (or develop) pain.

Methods for Treating Pain

[0032] In one aspect, the present disclosure is directed to a method for treating pain in a subject in need thereof. The method includes: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; identifying a difference in the expression level of the blood biomarker in the sample

and the reference expression level of the blood biomarker; and administering a treatment, wherein the treatment reduces the difference between the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker to mitigate pain in the subject.

[0033] The biomarkers are selected from the group listed in Tables 1, 4, 5, 7, and combinations thereof. In some embodiments, a panel of blood biomarkers is used. Biomarkers can be selected with different weighting coefficients possible.

[0034] Suitable treatments include those listed in Tables 1, 2, 7, and combinations thereof. Suitable treatments further include pain treatments known to those skilled in the art. Particularly suitable treatments include SC-560, pyridoxine, methylergometrine, LY-294002, haloperidol, cytisine, cyanocobalamin, apigenin, betaescin, amoxapine, and combinations thereof.

[0035] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is decreased as compared to the reference expression level of the biomarker.

[0036] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is increased as compared to the reference expression level of the biomarker.

[0037] In some embodiments, the method further includes performing a neuropsychological test on the subject. Generally, neuropsychological testing includes a comprehensive assessment of cognitive and personality functioning. More particularly, exemplary neuropsychological tests include: for intelligence (e.g., WAIS, WISC, SB, TONI); for achievement (e.g., WJ-III, WIAT, WRAT); for attention (e.g., CCPT, WCST, Vanderbilt, NEPSY); for language (e.g., GORT, Boston Naming, HRB-Aphasia for memory and learning (e.g., WMS, WRAML, CVLT, RAVLT, ROCF, NEPSY); for motor control (e.g., Grooved Pegoard, Finger Tapping, Grip Strength, Lateral Dominance); for visual (e.g., Spatial - ROCFT, Bender-Gestalt, HVOT); for autism (e.g., ADOS, ASDS, ADI, GARS); for executive functioning (e.g., WCST, BRIEF, EFSD, D-KEFS, HRB); and for behavioral (e.g., BASC, Achenbach, Vanderbilt).

Methods for Determining Pain

[0038] In one aspect, the present disclosure is directed to a method for determining High Pain intensity in a subject in need thereof. The method includes: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; and identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker.

[0039] As described herein, "Low Pain" refers to Visual Analog Scale (VAS) for pain of 2 and below; "Intermediate Pain" refers to VAS of 3-5; and "High Pain" refers to VAS of 6 and above (see, FIG. 3). The pain VAS is self-completed by the subject. The pain VAS is a continuous scale comprised of a horizontal (HVAS) or vertical (VVAS) line, usually 10 centimeters (100 mm) in length, anchored by 2 verbal descriptors, one for each symptom extreme (at 0 for "no pain" and at 100 for "worst imaginable pain"). The subject is asked to place a line perpendicular to the VAS line at the point that represents their pain intensity. Using a ruler, the score (i.e., intensity of pain) is determined by measuring the distance (mm) on the 10-cm line between the “no pain” anchor and the patient's mark, providing a range of scores from 0–100. A higher score indicates greater pain intensity.

[0040] While not used herein, other suitable pain tests include, for example, numeric rating scale (NRS), McGill Pain Questionnaire (MPQ), Short-form McGill Pain Questionnaire (SF-MPQ), Chronis Pain Grade Scale (CPGS), Short form 36 Bodily Pain Scale (SF-36 BPS), Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), and combinations thereof. For more information on these tests and applications thereof, *see* Hawker et al., Arthritis Care & Research, vol. 36, no. S11, November 2011, pp. S240-S252.

[0041] The biomarkers are selected from the group listed in Table 1, 4, 5, 7 and combinations thereof. In some embodiments, a panel of blood biomarkers is used. Biomarkers can be selected with different weighting coefficients possible.

[0042] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is increased as compared to the reference expression level of the biomarker.

[0043] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is decreased as compared to the reference expression level of the biomarker.

[0044] A particularly suitable biomarker for determining pain intensity is CNTN1.

[0045] In some embodiments, the subject is a female. A particularly suitable biomarker for predicting pain state in female subjects is DNAJC18.

[0046] In some embodiments, the subject is male. A particularly suitable biomarker for predicting pain state in female subjects is CTN1.

[0047] In some embodiments, the method further includes performing a neuropsychological test on the subject.

Methods for Predicting Future Medical Care Facility Visit for Pain

[0048] In another aspect, the present disclosure is directed to a method for predicting a future medical care facility visit for pain in a subject in need thereof. The method includes: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; and identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker, whereas the difference in the expression level of the blood biomarker in the sample obtained from the subject and the reference expression level of the blood biomarker determines the likelihood of future medical care facility/emergency department (ED) visits for pain.

[0049] As used herein, "emergency department (ED)" is used according to its ordinary meaning as understood by those skilled in the art to refer to medical care facilities specializing in emergency medicine, the acute care of patients who present without prior appointment; either by their own means or by that of an ambulance, and includes accident & emergency departments (A&E), emergency rooms (ER), emergency wards (EW) and casualty departments.

[0050] The biomarker is selected from the group listed in Table 1, 4, 5, 7 and combinations thereof. In some embodiments, a panel of blood biomarkers is used. Biomarkers can be selected with different weighting coefficients possible.

[0051] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is increased as compared to the reference expression level of the biomarker.

[0052] In some embodiments, the expression level of the blood biomarker in the sample obtained from the subject is decreased as compared to the reference expression level of the biomarker.

[0053] GBP1 is particularly suitable for predicting trait first year ED visits. GNG7 is particularly suitable for predicting trait all future ED visits.

[0054] In some embodiments, the subject is a female. GBP1 is particularly suitable as a predictor for trait first year ED visits in female subjects. ASTN2 is particularly suitable for trait all future ED visits in female subjects. When the subject is a female with bipolar disorder, CDK6 is a particularly suitable predictor for state. When the subject is a female with PTSD, SHMT1 is a particularly suitable predictor for trait first year ED visits. When the subject is a female with depression, GNG7 is a particularly suitable for trait all future ED visits.

[0055] In some embodiments, the subject is a male. CTN1 is particularly suitable as a predictor for state in male subjects. Hs.554262 is particularly suitable as a predictor for trait first year ED visits in male subjects. MFAP3 is particularly suitable for trait all future ED visits in male subjects. When the subject is a male with depression, CASPS is particularly suitable as a predictor for state. When the subject is a male with PTSD, LY9 is particularly suitable as a strong predictor for trait first year ED visits. When the subject is a male with PTSD MFAP3 is particularly suitable as a strong predictor for trait all future ED visits.

[0056] Particularly suitable biomarkers for pain include CCDC144B (Coiled-Coil Domain Containing 144B), COL2A1 (Collagen Type II Alpha 1 Chain), PPFIBP2 (PPFIA Binding Protein 2), DENND1B (DENN Domain Containing 1B), ZNF441 (Zinc Finger Protein 441), TOP3A (Topoisomerase (DNA) III Alpha), and ZNF429 (Zinc Finger Protein 429), and combinations thereof.

[0057] In some embodiments, the method further includes performing a neuropsychological test on the subject.

Prognosis of Pain

[0058] In another aspect, the present disclosure is directed to a method of prognosing pain in an individual in need thereof. As used herein, the term "prognosing" and "prognosis" are used according to their ordinary meaning as understood by those skilled in the art to refer to pain level increases from no pain to Low Pain to Moderate (Intermediate) Pain to High Pain.

[0059] The method includes: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; and identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker.

[0060] In some embodiments, the method further includes performing a neuropsychological test on the subject.

EXAMPLES

Materials and Methods

[0061] Three independent cohorts were used: discovery (major psychiatric disorders), validation (major psychiatric disorders with clinically severe pain disorders), and testing (an independent major psychiatric disorders cohort for predicting pain state, and for predicting future ER visits for pain) (see, FIG. 1A)

[0062] The psychiatric participants/subjects were part of a larger longitudinal cohort of adults that are being continuously collected. Participants were recruited from the patient population at the Indianapolis VA Medical Center. All participants understood and signed informed consent forms detailing the research goals, procedure, caveats and safeguards, per IRB approved protocol. Participants completed diagnostic assessments by an extensive structured clinical interview - Diagnostic Interview for Genetic Studies, and up to six testing visits, 3-6 months apart or whenever a new psychiatric hospitalization occurred. At each testing visit, the subject received a series of rating scales, including a visual analog scale (1-10) for assessing pain and the SF-36 quality of life scale, which has two pain related items (items 21 and 22), and blood was drawn. Whole blood (10 ml) was collected in two RNA-stabilizing PAXgene tubes, labeled with an anonymized ID number, and stored at -80 °C in a locked freezer until the time of future

processing. Whole-blood RNA was extracted for microarray gene expression studies from the PAXgene tubes, as detailed below.

[0063] For these Examples, the within-participant discovery cohort, from which the biomarker data were derived, consisted of 28 participants (19 males, 9 females) with multiple testing visits, who each had at least one diametric change in pain from Low Pain (VAS of 2 and below) to High Pain (VAS of 6 and above) from one testing visit to another (FIGS. 1B and 3). There were 3 participants with 5 visits each, 1 participants with 4 visits each, 12 participants with 3 visits each, and 12 participants with 2 visits each resulting in a total of 79 blood samples for subsequent gene expression microarray studies (FIGS. 1A-1C; Table 3).

[0064] The validation cohort, in which the top biomarker findings were validated for being even more changed in expression, consisted of 13 male and 10 female participants with a pain disorder diagnosis and clinically severe pain (Table 3). This was determined as having a pain VAS of 6 and above and a sum of SF36 scale items 21 (pain intensity) and 22 (impairment by pain of daily activities) of 10 and above. (*See*, Table 3).

[0065] The independent test cohort for predicting state (High Pain) consisted of 134 male and 28 female participants with psychiatric disorders, demographically matched with the discovery cohort, with one or multiple testing visits, with either Low Pain, intermediate Pain, or High Pain, resulting in a total of 414 blood samples in which whole-genome blood gene expression data were obtained (FIGS. 1A-1C and Table 3).

[0066] The test cohort for predicting trait (future ED visits with pain as the primary reason in the first year of follow-up, and all future ED visits for pain) (FIGS. 1A-1C) consisted of 171 males and 19 female participants for which longitudinal follow-up with electronic medical records were obtained. The participants' subsequent number of ED pain-related visits in the year following testing was tabulated from electronic medical records by a clinical researcher, who used the key word "pain" in the reasons for ED visit, or "ache" with a mention of acute pain in the text of the note.

[0067] Medications. The participants in the discovery cohort were all diagnosed with various psychiatric disorders, and had various medical co-morbidities (Table 1). Their medications were listed in their electronic medical records, and documented at the time of each testing visit. Medications can have a strong influence on gene expression. However, the

discovery of differentially expressed genes was based on within-participant analyses, which factored out not only genetic background effects, but also minimizes medication effects, as the participants rarely had major medication changes between visits. Moreover, there was no consistent pattern of any particular type of medication, as the participants were on a wide variety of different medications, psychiatric and non-psychiatric. Some participants may be non-compliant with their treatment and may thus have changes in medications or drug of abuse not reflected in their medical records. That being said, the goal was to discover biomarkers that track pain, regardless if the reason for it was endogenous biology or driven by substance abuse or medication non-compliance. In fact, one would expect some of these biomarkers to be targets of medications. Overall, the discovery of biomarkers with the universal design occurred despite the participants having different genders, diagnoses, being on various different medications, and other lifestyle variables.

Blood Gene Expression Experiments

[0068] RNA extraction. Whole blood (2.5–5 ml) was collected into each PaxGene tube by routine venipuncture. RNA was extracted and processed as previously described (see, Le-Niculescu, H. et al. Mol Psychiatry 18, 1249-64 (2013); Niculescu, A.B. et al. Mol Psychiatry 20, 1266-85 (2015); Levey, D.F. et al. Mol Psychiatry 21, 768-85 (2016)).

[0069] Microarrays. Microarray work was carried out as previously described (see, Le-Niculescu, H. et al. Mol Psychiatry 18, 1249-64 (2013); Niculescu, A.B. et al. Mol Psychiatry 20, 1266-85 (2015); Levey, D.F. et al. Mol Psychiatry 21, 768-85 (2016)).

Biomarkers

Step 1: Discovery.

[0070] The participant's score from the VAS Pain Scale was used, assessed at the time of blood collection (FIGS. 1A-1C). Gene expression differences between visits were analyzed with Low Pain (defined as a score of 0-2) and visits with High Pain (defined as a score of 6 and above), using a powerful within-participant design, then an across-participants summation (FIGS. 1A-1C).

[0071] Data was analyzed using an Absent-Present (AP) approach and a differential expression (DE) approach (see, Le-Niculescu, H. et al. Mol Psychiatry 18, 1249-64 (2013);

Niculescu, A.B. et al. Mol Psychiatry 20, 1266-85 (2015); Levey, D.F. et al. Mol Psychiatry 21, 768-85 (2016)). The AP approach can capture turning on and off of genes, and the DE approach can capture gradual changes in expression. R scripts were developed to automate and conduct all these large dataset analyses in bulk, checked against human manual scoring.

[0072] Gene symbol for the probe sets were identified using NetAffyx (Affymetrix) for Affymetrix HG-U133 Plus 2.0 GeneChips, followed by GeneCards to confirm the primary gene symbol. For those probesets that were not assigned a gene symbol by NettAffyx, GeneAnnot was used to obtain gene symbols for the uncharacterized probesets, followed by GeneCard. Genes were then scored using a manually curated CFG database as described below (FIG. 1E).

Step 2. Prioritization using Convergent Functional Genomics (CFG).

[0073] Databases. 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, were created. Only findings deemed significant in the primary publication, by the study authors, using their particular experimental design and thresholds were included in the databases. The databases included only primary literature data and did not include review papers or other secondary data integration analyses to avoid redundancy and circularity. These large and constantly updated databases have been used in the inventors' CFG cross validation and prioritization platform (FIG. 1E). For these Examples, data from 355 papers on pain were present in the databases at the time of the CFG analyses (December 2017) (human genetic studies-212, human nervous tissue studies-3, human peripheral tissue/fluids- 57, non-human genetic studies-26, non-human brain/nervous tissue studies-48, non-human peripheral tissue/fluids- 9). Analyses were performed as described herein and in Le-Niculescu, H. et al. Mol Psychiatry 18, 1249-64 (2013); Niculescu, A.B. et al. Mol Psychiatry 20, 1266-85 (2015); Levey, D.F. et al. Mol Psychiatry 21, 768-85 (2016).

Step 3. Validation analysis.

[0074] Validation analyses of candidate biomarker genes were conducted separately for AP and for DE. Which of the top candidate genes (total CFG score of 6 or above), were stepwise changed in expression from the Low Pain and High Pain group to the Clinically Severe Pain

group was determined. A CFG score of 6 or above reflected an empirical cutoff of 33.3% of the maximum possible CFG score of 12, which permitted the inclusion of potentially novel genes with maximal internal score of 6 but no external evidence score. Participants with Low Pain, as well as participants with High Pain from the discovery cohort who did not have severe clinical pain (SF36 sum of item 21 and 22 <10) were used, along with the independent validation cohort which all had severe clinical pain and a co-morbid pain disorder diagnosis (n= 23).

[0075] For the AP analysis, the Affymetrix microarray .chp data files from the participants in the validation cohort of severe pain were imported into MAS5 Affymetrix Expression Console, alongside the data files from the Low Pain and High Pain groups in the live discovery cohort. The AP data was transferred to an Excel sheet and A was transformed into 0, M into 0.5 and P into 1. Everything was Z-scored together by gender and diagnosis. If a probe set would have shown no variance, and thus, gave a non-determined (0/0) value in Z-scoring in a gender and diagnosed, the value was excluded from the analysis for that probeset for that gender and diagnosis from the analysis.

[0076] For the DE analysis, the cohorts were assembled out of Affymetrix .cel data that was RMA normalized by gender and diagnosis. The log transformed expression data was transferred to an Excel sheet, and non-log data transformed by taking 2 to the power of the transformed expression value. The values were then Z-scored by gender and diagnosis.

[0077] The Excel sheets with the Z-scored by gender and diagnosis AP and DE expression data were imported into Partek, and statistical analyses were performed using a one-way ANOVA for the stepwise changed probesets, and a stringent Bonferroni corrections were performed for all the probesets tested in AP and DE (stepwise and non-stepwise) (FIG. 1F). An R script that automatically analyzes the data directly from the Excel sheet was then developed and used to confirm the calculations.

Choice of biomarkers to be carried forward

[0078] The top biomarkers from each step were carried forward. The longer list of candidate biomarkers includes the top biomarkers from discovery step (>=90% of scores, n=28), the top biomarkers from the prioritization step (CFG score >=8, n=32), and the nominally significant biomarkers after the validation step (n=5), for a total of n= 65 probesets (n=60 genes). The short list of top biomarkers after the validation step is 5 biomarkers. In Step 4 testing,

prediction with the biomarkers from the long list in independent cohorts High Pain state, and future ED visits for pain in the first year, and in all future years were performed.

Diagnostics

[0079] The test cohort for predicting High Pain (state), and the subset of it that was a test cohort for predicting future ER visits (trait), were assembled out of data that was RMA normalized by gender and diagnosis. The cohort was completely independent, as there was no subject overlap with the discovery cohort. Phenomic (clinical) and gene expression markers used for predictions were Z-scored by gender and diagnosis to be able to combine different markers into panels and to avoid potential artifacts due to different ranges of expression in different gender and diagnoses. Markers were combined by simple summation of the increased risk markers minus the decreased risk markers. Predictions were performed using R studio.

[0080] Predicting High Pain State. Receiver-operating characteristic (ROC) analyses between genomic and phenomic marker levels and Pain were performed by assigning participants with a Pain score of 6 and greater into the High Pain category. The pROC package of R (Xavier Robin et al. BMC Bioinformatics 2011) was used. The z-scored biomarker and phene scores were run in the ROC generating program against the diagnostic groups in the independent test cohort (High Pain vs. the rest of participants). Additionally, a one-tailed t-test was performed between High Pain group versus the rest, and Pearson R (one-tail) was calculated between Pain scores and marker levels.

[0081] Predicting Future ER visits for Pain in First Year Following Testing. Analysis for predicting ER visits for Pain in the first year following each testing visit in subjects that had at least one year of follow-up in the VA system was conducted. ROC analysis between genomic and phenomic marker levels at specific testing visit and future ER visits for Pain were performed as previously described based on assigning if participants had visited the ER with primary reason for Pain or not within one year following a testing visit. Additionally, a one tailed t-test with unequal variance was performed between groups of participant visits with and without ER visits for pain. Person R (one-tail) correlation was performed between hospitalization frequency (number of ER visits for pain divided by duration of follow-up) and marker levels. A Cox regression was performed using the time in days from the testing visit date to first ER visit date in the case of patients who had been to the ER, or 365 days for those who did not. The hazard

ratio was calculated such that a value greater than 1 always indicated increased risk for ER visits, regardless if the biomarker was increased or decreased in expression.

[0082] Odds ratio analysis was conducted for ER visits for pain for all future ER visits due to pain, including those occurring beyond one year of follow-up, in the years following testing (on average 5.26 years per participant, range 0.44 to 11.27 years; see Tables 1 and 3), as this calculation, unlike the ROC and t-test, accounts for the actual length of follow-up, which varied from participant to participant. Without being bound by theory, the ROC and t-test may, if used, under-represent the power of the markers to predict, as the more severe psychiatric patients are more likely to move geographically and/or be lost to follow-up. A Cox regression was also performed using the time in days from visit date to first ER Pain visit date in the case of patients who had been to the ER for pain, or from visit date to last note date in the electronic medical records for those who did not. The hazard ration was calculated such that a value greater than 1 always indicated increased risk for ER Pain related visits, regardless if the biomarker was increased or decreased in expression.

Biological Understanding

Pathway analysis

[0083] IPA (Ingenuity Pathway Analysis, version 24390178, Qiagen), David Functional Annotation Bioinformatics Microarray Analysis (National Institute of Allergy and Infectious Diseases) version 6.7 (August 2016), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (through DAVID) were used to analyze the biological roles, including top canonical pathways and diseases (Table 6), of the candidate genes resulting from these Examples, as well as to identify genes in the dataset that were the target of existing drugs. The pathway analysis for the combined AP and DE probesets identified 60 unique genes (65 probesets). Network analysis of the 60 unique genes was performed using STRING Interaction Network by in putting the genes into the search window and performing Multiple Proteins Homo sapiens analysis.

CFG beyond Pain: evidence for involvement in other psychiatric and related disorders.

[0084] A CGF approach was also used to examine evidence from other psychiatric and related disorders for the list of 65 candidate biomarkers (Table 5).

Therapeutics

[0085] Pharmacogenomics. Which of the individual top biomarkers were analyzed for knowing to be modulated by existing drugs using the CFG databases and using Ingenuity Drugs analysis (Table 7).

[0086] New drug discovery/repurposing. Drugs and natural compounds were also analyzed as an opposite match for the gene expression profile of panels of the top biomarkers (n=65) using the Connectivity Map (Broad Institute, MIT) (Table 2). 33 of 65 probesets were present in the HGU-133A array used for the Connectivity Map. The NIH LINCS L1000 database was also used (Table 4).

Convergent Functional Evidence

[0087] All the evidence from discovery (up to 6 points), prioritization (up to 12 points), validation (up to 6 points), testing (state, trait first year ED visits, trait all future ED visits- up to 8 points each if significantly predicts in all participants, 6 points if predicts by gender, 4 points if predicts in gender/diagnosis) were tabulated into a convergent functional evidence score. The total score could be up to 48 points: 36 from this data and 12 from literature data. The data from these Examples were weighed three times as much as the literature data. The Examples highlight, based on the totality of the experimental data and of the evidence in the field to date, biomarkers having all around evidence: those that tracked pain, those that predicted it, those that were reflective of pain and other pathology, and those that were potential drug targets.

[0088] Provided herein is a powerful longitudinal within-participant design in individuals with psychiatric disorders to discover blood gene expression changes between self-reported Low Pain and High Pain states (FIGS. 1A-1C). A longitudinal within-participant design is orders of magnitude more powerful than a cross-sectional case-control design. Some of these candidate gene expression biomarkers are increased in expression in High Pain states (being putative risk genes, or "algogenes"), and others are decreased in expression (being putative protective genes, or "pain suppressor genes").

[0089] The list of candidate biomarkers was prioritized with a Bayesian-like Convergent Functional Genomics approach, comprehensively integrating previous human and animal model evidence in the field.

[0090] The top biomarkers from discovery and prioritization were validated in an independent cohort of psychiatric subjects carrying a diagnosis of a pain disorder and with high scores on pain severity ratings. A list of 65 candidate biomarkers (Tables 1 and 3), including a shorter list of 5 validated biomarkers (MFAP3, PIK3CD, SVEP1, TNFRSF11B, ELAC2) was obtained from the first three steps. The biomarkers with the best evidence after validation were Hs.666804/MFAP3 (p=6.03E-04) and PIK3CD (p=1.59E-02).

[0091] The 65 candidate biomarkers were analyzed for predicting pain severity state and future emergency department (ED) visits for pain in another independent cohort of psychiatric subjects. The biomarkers were analyzed in all subjects in the test cohort, as well as by gender and psychiatric diagnosis, which showed increased accuracy, particularly in women (FIG. 2). In general, the longitudinal information was more predictive than the cross-sectional information. Across all participants tested, CNTN1 was the best predictor for state (AUC 63%, p=0.0014), GBP1 the best predictor for trait first year ED visits (AUC 59%, p=0.0035), and GNG7 the best predictor for trait all future ED visits (OR 1.28, p=0.000161, surviving Bonferroni correction for the 65 biomarkers tested). By gender, in females, DNAJC18 was the best predictor for state (AUC 78%, p=0.0049), GBP1 the best predictor for trait first year ED visits (AUC 71%, p=0.043) and ASTN2 for trait all future ED visits (OR 2.45, p=0.043). In males, CNTN1 was the best predictor for state (AUC 63%, p=0.0022), Hs.554262 the best predictor for trait first year ED visits (AUC 59%, p=0.016), and MFAP3 the best predictor for trait all future ED visits (OR 1.34, p=0.014). Personalized by gender and diagnosis, in female bipolar, CDK6 was a strong predictor for state (AUC 100%, p=0.007), in female PTSD, SHMT1 was a strong predictor for trait first year ED visits (AUC 100%, p=0.022), and in female depression GNG7 for trait all future ED visits (OR 14.54, p=0.023). In male depression, CASPS was a strong predictor for state (AUC 87%, p=0.00007, surviving Bonferroni correction for the 65 biomarkers tested), in male PTSD, LY9 was a strong predictor for trait first year ED visits (AUC 77%, p= 0.041), and in male PTSD, MFAP3 was a strong predictor for trait all future ED visits (OR 15.95, p=0.00084). Predictions of future ED visits for pain in the independent cohorts were consistently stronger using biomarkers than clinical phenotypic markers (pain VAS scale, pain items 21 and 22 from SF-36), supporting the utility of biomarkers. Also, in general, panels of all 65 biomarkers or of the 5 validated biomarkers did not work as well as individual biomarkers, particularly when the later are tested by gender and diagnosis, consistent with there being heterogeneity in the population and supporting the need for personalization. The notable

exception was predicting all future ED visits for pain, where the panel of 5 validated biomarkers performed better than individual biomarkers.

[0092] The biomarkers were further analyzed for involvement in other psychiatric and related disorders (Table 5). A majority of the biomarkers have some evidence in other disorders, whereas a few seemed to be specific for pain, such as CCDC144B (Coiled-Coil Domain Containing 144B), COL2A1 (Collagen Type II Alpha 1 Chain), PPFIBP2 (PPFIA Binding Protein 2), DENND1B (DENN Domain Containing 1B), ZNF441 (Zinc Finger Protein 441), TOP3A (Topoisomerase (DNA) III Alpha), and ZNF429 (Zinc Finger Protein 429). A majority of the biomarkers (50 out of 60 genes, i.e. 83.3%) have prior evidence for involvement in suicide, indicating an extensive molecular co-morbidity between pain and suicide, to go along with the clinical and phenomenological co-morbidity (physical pain, psychic pain). The biological pathways and networks the biomarkers are involved in were analyzed (Table 6 and FIG. 4). There was a network centered on GNG7 (FIG. 4), that may be involved in connectivity/signaling, comprising HTR2A, EDN1, PNOC (involved in pain signaling) and CALCA (involved in Reflex Sympathetic Dystrophy and Complex Regional Pain Syndrome). It was reassuring that PNOC (Prepronociceptin) increased in expression in high pain states, i.e. as an algogene. Given its known roles in pain, it can serve as a *de facto* positive control. A second network was centered on CCND1, may be involved in activity/trophicity, and comprises HRAS, CDK6, PBRM1, CSDA, LOXL2, EDN1, PIK3CD, and VEGFA. A third network was centered on HLA DRB1, may be involved in reactivity/immune response, and comprises GBP1, ZNF429, COL2A1, and HLA DQB1, from the list of 65 top biomarkers.

[0093] The biomarkers were analyzed as targets of existing drugs and thus could be used for pharmacogenomics population stratification and measuring of response to treatment (Table 7), as well as used the biomarker gene expression signature to interrogate the Connectivity Map database from Broad/MIT to identify drugs and natural compounds that can be repurposed for treating pain (Table 2). The top drugs identified as potential new pain therapeutic were SC-560, an NSAID, haloperidol, an antipsychotic, and amoxapine, an antidepresseant. The top natural compounds were pyridoxine (vitamin B6), cyanocobalamin (vitamin B12), and apigenin (a plant flavonoid).

[0094] The biomarkers with the best overall evidence across the six steps were GNG7, CNTN1, LY9 CCDC144B, GBP1 and MFAP3 (Table 1). GNG7 (G Protein Subunit Gamma 7) was decreased in expression in blood in High Pain states, i.e., it is a pain suppressor gene. There

is evidence in other tissues in human studies for involvement in pain (diabetic neuropathy, vertebral disc). GNG7 also has trans-diagnostic evidence for involvement in other psychiatric disorders. It is decreased in expression in mouse brain by alcohol, hallucinogens, and stress, and increased in expression by omega-3 fatty acids. CNTN1 (Contactin 1) was decreased in expression in blood in High Pain states, i.e. it is a pain suppressor gene. Reassuringly, there was convergent evidence in other tissues in human studies for involvement in pain: CNTN1 has also been reported to be decreased in expression in CSF in women with chronic widespread pain (CWP). Anti-contactin 1 autoantibodies, that block/decrease levels of contactin 1, have been described in chronic inflammatory demyelinating polyneuropathy4. CNTN1 has also trans-diagnostic evidence for involvement in psychiatric disorders. It is decreased in expression in schizophrenia brain and blood, and in blood in suicidality in females. CNTN1 was increased in expression by clozapine in mouse brain. LY9 (Lymphocyte Antigen 9) was increased in expression in blood in High Pain states, i.e. it is an algogene. It also has epigenetic evidence for involvement in exposure to stress, and is decreased in expression by omega-3 fatty acids in mouse brain. CCDC144B (Coiled-Coil Domain Containing 144B) was decreased in expression in blood in High Pain states. There is evidence in other tissues in human and animal model studies for involvement in pain. CCDC144B was a good predictor in the independent cohorts for state and trait, particularly for males with psychosis (SZ, SZA). It does not have trans-diagnostic evidence for involvement in other psychiatric disorders, seeming to be relatively specific for pain. GBP1 (Guanylate Binding Protein 1), with interferon induced signaling roles, is increased in expression in blood in High Pain states. There is other evidence in human studies, gene expression and genetic, for involvement in pain. GBP1 is a predictor in the independent cohorts for trait, particularly in females. It is increased in expression in the brain in MDD, schizophrenia, and suicide, and in blood in PTSD. GBP1 was decreased in expression by omega-3 in mouse brain. Hs.666804/MFAP3 (Microfibril Associated Protein 3), another of the top markers, is a component of elastin-associated microfibrils. MFAP3 had the most robust empirical evidence from the discovery and validation steps, and was a strong predictor in the independent cohort, particularly for pain in females and males with PTSD. Interestingly, it has no prior evidence for pain in the literature curated to date for the Prioritization/CFG step, which demonstrates that a wide-enough net was cast with the disclosed approach that can bring to the fore completely novel findings. MFAP3 was decreased in expression in blood in High Pain states, i.e., it is a pain suppressor gene. It also has previous evidence for involvement in alcoholism, stress, and suicide.

[0095] As disclosed herein, clustering analysis of a discovery cohort composed of participants with psychiatric disorders followed longitudinally over time, in which each participant had blood samples collected and neuropsychological testing done in at least one low pain state visit (Pain VAS $\leq$ 2 out of 10) and at least one high pain state visit (Pain VAS $\geq$6 out of 10), revealed two broad subtypes of high pain states: a predominantly psychotic subtype, possibly related to mis-connectivity and increased perception of pain centrally, and a predominantly anxious subtype, possibly related to reactivity and increased physical health reasons for pain peripherally. The powerful longitudinal within-participant design was used to discover blood gene expression changes between self-reported low pain and high pain states. Some of these gene expression biomarkers were increased in expression in high pain states (being putative risk genes, or "algogenes"), and others were decreased in expression (being putative protective genes, or "pain suppressor genes").

[0096] Advantageously, the present disclosure enables precision medicine for pain, with objective diagnostics and targeted novel therapeutics. Given the massive negative impact of untreated pain on quality of life, the current lack of objective measures to determine appropriateness of treatment, and the severe addiction gateway potential of existing opioid-based pain medications, the present disclosure provides herein. The methods described herein provide objective biomarkers for pain, which is a subjective sensation. Further, the biomarkers provided herein are able to objectively determine pain state and predict future emergency department visits for pain, even more so when personalized by gender and diagnosis. The biomarkers are suitable for targeting using existing drugs and yielded new drug candidates.

[0097] In view of the above, it will be seen that the several advantages of the disclosure are achieved and other advantageous results attained. As various changes could be made in the above methods and systems without departing from the scope of the disclosure, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[0098] When introducing elements of the present disclosure or the various versions, embodiment(s) or aspects thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Table 1. Convergent Functional Evidence (CFE) for Top Candidate Biomarkers for Pain (n=60 genes, 65 probesets).

Gene Symbol/ Gene Name	Probesets	Step 1 Discovery in Blood (Direction of Change) Method/ Score/ % Up to 6pts	Step 2 External Convergent Functional Genomics (CFG) Evidence For Involvement in Pain Score Up to 12pts	Step 3 Validation in Blood ANOVA p-value/ Score Up to 6 pts	Step 4 Best Significant Prediction of State- High Pain (Cases/Total) ROC AUC/ p-value 8 pts All 6pts Gender 4pts Gender/Dx	Step 4 Best Significant Prediction of Trait-Future ED visits for Pain in the first year (Cases/Total) ROC AUC/ p-value 8 pts All 6pts Gender 4pts Gender/Dx	Step 4 Best Significant Predictions of Trait-Future ED visits for Pain in all future years (Cases/Total) OR/OR p-value 8 pts All 6pts Gender 4pts Gender/Dx	Step 5 Other Psychiatric and Related Disorders Evidence	Step 6 Drugs that Modulate the Biomarker in Opposite Direction to Pain	CFE Polyevidence Score for Involvement in Pain (Based on Steps 1-4)
GNG7 G Protein Subunit Gamma 7	1566643_a_at	(D) DE/4 59%	6	6.81E-02/2 Stepwise	**All** **C:**(101/411) 0.56/3.52E-02 **Gender** Male **C:**(85/346) 0.56/3.95E-02 **Gender/Dx** M-SZ **C:**(11/64) 0.68/2.79E-02	**Gender** Females **C:**(7/44) 0.7/4.92E-02 **Gender/Dx** F-MDD **C:**(4/11) 0.82/4.45E-02 **L:**(2/6) 1/3.20E-02 F-PTSD **C:**(2/8) 0.92/4.78E-02	**All** **C:**(239/501) 1.28/**1.03E-04**** **L:**(145/309) 1.22/1.70E-02 **Gender** Females **C:**(13/47) 1.69/4.69E-02 Males **C:**(226/454) 1.28/**1.92E-04**** **L:**(138/282) 1.21/2.16E-02 **Gender/Dx** F-MDD	Alcohol BP Hallucinogens MDD Stress SZ	Omega-3 fatty acids	34

CNTN1 Contactin 1	1554784_at	(D) DE/4 52%	6	NS	**All** **C:**(101/411) 0.58/1.15E-02 **L:**(61/248) 0.63/1.42E-03 **Gender** Female **C:**(16/65) 0.65/3.38E-02 Male **L:**(51/212) 0.63/2.27E-03 **Gender/Dx** M-BP **C:**(24/123) 0.61/4.13E-02 **L:**(16/81) 0.64/4.06E-02 M-SZ	**Gender** Males **C:**(95/426) 0.56/3.08E-02	**Gender/Dx** M-MDD **C:**(42/72) 1.44/1.23E-02 **L:**(25/43) 1.64/4.17E-02	BP MDD SZ Suicide	Clozapine	28
							C:(4/12) 14.54/2.23E-02 M-MDD **L:**(25/43) 1.8/2.70E-02 M-PSYCHOSIS **C:**(95/201) 1.52/**1.70E-04**** **L:**(57/120) 1.34/2.47E-02 M-SZ **C:**(42/103) 1.58/2.08E-02 M-SZA **C:**(53/98) 1.71/**4.40E-04****			

					C:(11/64) 0.68/3.15E-02 M-MDD **L:**(13/43) 0.66/4.53E-02 M-SZA **L:**(3/17) 0.83/3.89E-02				
LY9 Lymphocyte Antigen 9	231124_x_at	(I) DE/6 90%	2	NS	**All** **C:**(101/411) 0.56/4.40E-02 **L:**(61/248) 0.58/2.39E-02 **Gender** Male **C:**(85/346) 0.57/3.02E-02 **L:**(51/212) 0.62/5.19E-03 **Gender/Dx** M-BP **C:**(24/123) 0.63/2.66E-02 F-MDD **C:**(2/18) 0.97/1.75E-02 M-MDD **L:**(13/43) 0.8/9.87E-04	**All** **C:**(102/470) 0.56/2.30E-02 **Gender** Males **C:**(95/426) 0.59/2.61E-03 **Gender/Dx** M-BP **C:**(18/120) 0.68/6.91E-03 M-PTSD **L:**(10/16) 0.77/4.13E-02	**Gender/Dx** M-MDD **C:**(42/72) 1.65/3.85E-03 **L:**(25/43) 1.53/3.74E-02 M-PTSD **L:**(18/20) 2.07/6.77E-03	Acute Stress	Omega-3 fatty acids	28
CCDC144B Coiled-Coil Domain Containing 144B (Pseudogene)	1557366_at	(D) DE/4 56%	6	NS	**Gender/Dx** F-BP **C:**(4/21) 0.79/3.66E-02	**Gender/Dx** M-MDD **C:**(26/67)	**All** **C:**(239/501) 1.23/2.27E-03			26

GBP1 Guanylate Binding Protein 1	231578_at	(I) DE/2 37%	6	3.26E-01/2 Stepwise	M-PSYCHOSIS **C:**(19/96) 0.68/8.95E-03 **L:**(10/56) 0.68/4.16E-02 M-SZA **L:**(3/17) 0.9/1.61E-02	0.63/3.43E-02	**Gender** Males **C:**(226/454) 1.23/3.34E-03 **Gender/Dx** M-PSYCHOSIS **C:**(95/201) 1.41/3.46E-03 **L:**(57/120) 1.43/1.32E-02 M-SZ **C:**(42/103) 1.84/4.65E-03 M-SZA **L:**(32/56) 1.47/3.49E-02	MDD PTSD SZ	Omega-3 fatty acids	26
						All **C:**(102/470) 0.59/3.51E-03 **Gender** Females **C:**(7/44) 0.71/4.30E-02 Males **C:**(95/426) 0.58/1.04E-02 **Gender/Dx** F-MDD **C:**(4/11) 0.93/1.17E-02 M-PSYCHOSIS **C:**(33/198) 0.6/3.25E-02 M-SZA	**All** **C:**(239/501) 1.09/3.72E-02 **Gender** Females **C:**(13/47) 1.68/2.41E-02 **Gender/Dx** F-MDD **C:**(4/12) 3.1/4.43E-02 M-SZA **C:**(53/98) 1.22/3.65E-02		

Hs.666804/ MFAP3 Microfibril Associated Protein 3	240949_x_at	(D) DE/6 81%	0	6.03E-04/4 Nominal	**Gender/Dx** F-PTSD **C:**(5/12) 0.8/4.41E-02	**C:**(23/97) 0.62/4.10E-02	**Gender/Dx** M-BP L:(9/80) 0.75/7.27E-03	**All** **L:**(145/309) 1.28/2.28E-02 **Gender** Males **C:**(226/454) 1.17/2.64E-02 **L:**(138/282) 1.35/8.94E-03 **Gender/Dx** M-BP **L:**(34/91) 2.36/**4.86E-04**** M-PTSD **L:**(18/20) 15.93/8.46E-04	Alcohol Suicide Stress	26
CASP6 Caspase 6	209790_s_at	(I) DE/4 51%	4	NS	**Gender** Male **L:**(51/212) 0.59/2.92E-02 **Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02 M-MDD **L:**(13/43) 0.87/**7.01E-05****	**Gender** Males **C:**(95/426) 0.57/2.54E-02 **Gender/Dx** M-PSYCHOSIS **C:**(33/198) 0.6/2.88E-02 M-SZA **C:**(23/97) 0.63/2.71E-02	**Gender/Dx** M-MDD **C:**(42/72) 1.31/3.97E-02	BP	24
COMT Catechol-O-Methyltransferase	216204_at	(D) DE/4 54%	4	NS	**Gender/Dx** M-MDD **L:**(13/43) 0.71/1.41E-02	**All** **C:**(102/470) 0.55/4.48E-02 **Gender** Males	**Gender/Dx** M-BP **L:**(34/91) 1.65/2.20E-02	ADHD Aggression Alcohol Anxiety BP	Clozapine Morphine Mood Stabilizers	24

					C:(95/426) 0.57/1.95E-02 **Gender/Dx** M-MDD C:(26/67) 0.66/1.58E-02 M-PSYCHOSIS C:(33/198) 0.6/3.63E-02		Chronic Stress MDD OCD Panic Disorder Psychosis PTSD Suicide SZ		
RAB33A RAB33A, Member RAS Oncogene Family	206039_at	(I) DE/6 90%	0	NS	**Gender/Dx** F-MDD C:(2/18) 1/1.23E-02	**Gender** Males C:(95/426) 0.56/3.60E-02	**All** C:(239/501) 1.14/2.21E-02 **Gender** Males C:(226/454) 1.16/1.01E-02 **Gender/Dx** M-BP L:(34/91) 1.65/1.69E-03 M-MDD C:(42/72) 1.95/**6.59E-04**** L:(25/43) 1.85/1.72E-02	Alcohol Stress MDD	24
ZYX Zyxin	238016_s_at	(D) DE/4 57%	4	NS	**Gender/Dx** F-BP C:(4/21) 0.78/4.44E-02	**All** C:(102/470) 0.55/4.80E-02 **Gender** Males C:(95/426) 0.57/1.58E-02 **Gender/Dx**	**Gender/Dx** M-BP L:(34/91) 1.85/1.67E-02 M-PTSD C:(26/31) 1.57/4.40E-02 L:(18/20) 2.2/1.53E-02	MDD	Clozapine 24

					M-PSYCHOSIS C:(33/198) 0.62/1.43E-02 M-SZA C:(23/97) 0.66/1.15E-02 M-BP L:(9/80) 0.71/2.26E-02				
(Hs.696420) MTERF1 Mitochondrial Transcription Termination Factor 1	243125_x_at	(D) DE/6 100%	0	NS	**Gender/Dx** M-PSYCHOSIS **C:**(19/96) 0.67/1.01E-02 M-SZ **C:**(11/64) 0.77/2.27E-03 **L:**(7/39) 0.71/3.95E-02	**Gender/Dx** F-PTSD **C:**(2/8) 1/2.28E-02	**All** **C:**(239/501) 1.19/1.19E-02 **L:**(145/309) 1.2/4.81E-02 **Gender** Males **C:**(226/454) 1.19/1.51E-02 **Gender/Dx** M-PSYCHOSIS **C:**(95/201) 1.41/8.86E-03 M-SZ **C:**(42/103) 1.4/4.47E-02 M-SZA **C:**(53/98) 1.44/4.72E-02	PTSD Suicide	22
COL27A1 Collagen Type XXVII Alpha 1 Chain	225293_at	(D) DE/4 79%	4	7.47E-01/2 Stepwise	**Gender/Dx** M-MDD **L:**(13/43) 0.66/4.79E-02	**Gender/Dx** M-MDD **C:**(26/67) 0.63/3.38E-02 M-PSYCHOSIS **C:**(33/198)	**Gender/Dx** M-PTSD **L:**(18/20) 1.96/2.37E-02	Tourette syndrome	Lithium	22

					0.61/2.79E-02 M-SZA **C:**(23/97) 0.68/4.96E-03 **L:**(13/55) 0.7/1.62E-02				
HRAS HRas Proto-Oncogene, GTPase	212983_at	(I) DE/6 97%	0	NS	**All** **C:**(101/411) 0.56/3.47E-02 **L:**(61/248) 0.58/3.01E-02 **Gender** Male **C:**(85/346) 0.57/2.72E-02 **L:**(51/212) 0.61/1.18E-02 **Gender/Dx** M-SZ **C:**(11/64) 0.68/2.79E-02 M-MDD **L:**(13/43) 0.71/1.61E-02	**Gender/Dx** F-PTSD **C:**(2/8) 1/2.28E-02	**Gender/Dx** M-MDD **C:**(42/72) 2.2/**3.38E-06**** **L:**(25/43) 2.25/**2.61E-04****	Alcohol BP Longevity Suicide SZ	ISIS 2503	22
CALCA Calcitonin Related Polypeptide Alpha	210727_at	(D) DE/4 54%	7	NS	**Gender** Females **C:**(16/63) 0.66/3.12E-02 **Gender/Dx** F-MDD **C:**(2/18) 0.97/1.75E-02	**Gender/Dx** F-PTSD **C:**(2/8) 1/2.28E-02 Gender/Dx M-PSYCHOSIS **C:**(33/198) 0.6/3.87E-02		Alcohol Anxiety Panic Disorder	Omega-3 fatty acids Lithium	21

					F-BP L:(3/11) 0.88/3.31E-02 M-MDD L:(13/43) 0.66/4.79E-02					
(Hs.596713) **PPP1R14B** Protein Phosphatase 1 Regulatory Inhibitor Subunit 14B	226138_s_at	(D) DE/6 90%	0	6.28E-02/2 Stepwise	**Gender/Dx** F-BP **C:**(4/21) 0.94/3.61E-03 **L:**(3/11) 0.92/2.06E-02 M-MDD **L:**(13/43) 0.73/9.98E-03		**All** **C:**(239/501) 1.15/1.43E-02 **Gender** Males **C:**(226/454) 1.19/4.84E-03 **L:**(138/282) 1.2/3.94E-02 **Gender/Dx** M-PSYCHOSIS **C:**(95/201) 1.35/3.06E-03 M-SZ **C:**(42/103) 1.53/3.19E-02 M-SZA **C:**(53/98) 1.41/9.26E-03	SZ	Lithium	20
						Gender/Dx F-MDD **L:**(2/6) 1/3.20E-02	**Gender** Female **L:**(7/27) 2.45/4.36E-02	Suicide SZ ASD BP MDD	Antipsychotics	20
ASTN2 Astrotactin 2	1554816_at	(I) DE/6 83%	2	1.71E-01 /2 Stepwise						
ELAC2 ElaC Ribonuclease Z 2	201766_at	(D) DE/4 52%	2	4.11E-02/4 Nominal	**Gender/Dx** M-MDD **L:**(13/43)		**Gender** Males **L:**(138/282)	ASD		20

					1.2/4.61E-02 **Gender/Dx** M-BP **L:**(34/91) 1.55/4.79E-02 M-MDD **C:**(42/72) 1.69/2.47E-03 **L:**(25/43) 1.85/3.66E-02	Alcohol Depression Longevity Stress Suicide SZ	Antipsychotics	20	
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	212998_x_at	(I) DE/4 51%	8	NS	0.73/8.66E-03 **Gender/Dx** M-SZ **C:**(11/64) 0.68/3.41E-02 F-MDD **C:**(2/18) 1/1.23E-02 M-MDD **L:**(13/43) 0.67/4.28E-02	**Gender/Dx** M-BP **L:**(34/91) 1.63/1.30E-02	Alcohol Depression Longevity Stress Suicide SZ	Antipsychotics	20
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211656_x_at	(I) DE/4 59%	8	NS	**Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02 M-SZ **C:**(11/64) 0.68/3.15E-02 M-SZ **C:**(11/64) 0.74/5.90E-03 **L:**(7/39) 0.72/3.36E-02	**Gender/Dx** M-MDD **C:**(26/67) 0.62/4.85E-02	Alcohol BP Depression Longevity PTSD Stress Suicide SZ	Antipsychotics	20

PNOC Preprononociceptin	205901_at	(I) DE/4 62%	4	NS	M-MDD L:(13/43) 0.69/2.68E-02 M-PSYCHOSIS L:(10/56) 0.69/3.29E-02	**Gender/Dx** M-BP L:(9/80) 0.68/4.20E-02	**Gender/Dx** M-BP **C:**(53/134) 1.23/4.73E-02 **L:**(34/91) 1.26/2.67E-02 M-MDD **C:**(42/72) 1.4/2.09E-02	Addictions BP MDD SZ Stress	20
TCF15 Transcription Factor 15 (Basic Helix-Loop-Helix)	207306_at	(D) DE/6 94%	2	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.94/2.46E-02 M-MDD **L:**(13/43) 0.68/3.21E-02		**All** **C:**(239/501) 1.11/4.85E-02 **Gender** Males **C:**(226/454) 1.14/2.39E-02 **Gender/Dx** M-BP **L:**(34/91) 2.22/2.61E-03	Suicide	20
TOP3A Topoisomerase (DNA) III Alpha	214300_s_at	(D) DE/4 51%	4	NS	**Gender/Dx** F-BP **C:**(4/21) 0.84/1.97E-02		**All** **L:**(145/309) 1.18/4.66E-02 **Gender** Males **L:**(138/282) 1.2/3.88E-02	Omega-3 fatty acids	20

(H05785) **LRRC75A** Leucine Rich Repeat Containing 75A	236913_at	(D) AP/6 97%	0	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.94/2.46E-02	**All** **C:**(102/470) 0.56/2.27E-02 L:(58/287) 0.58/3.38E-02 **Gender** Males **C:**(95/426) 0.57/1.64E-02 L:(54/261) 0.59/2.71E-02 **Gender/Dx** F-PTSD **C:**(2/8) 1/2.28E-02 M-PSYCHOSIS **C:**(33/198) 0.65/3.29E-03 M-SZA **C:**(23/97) 0.68/5.21E-03 M-SZA **L:**(13/55) 0.66/4.42E-02 M-MDD L:(16/39)	**Gender/Dx** M-SZ **L:**(25/64) 1.75/4.72E-02	Alcohol BP Suicide SZ	Clozapine	18

CLSPN Claspin	242150_at	(I) AP/6 95%	0	NS	**Gender/Dx** M-PSYCHOSIS **C:**(19/96) 0.65/2.48E-02	0.76/3.64E-03		Suicide		18
						All L:(58/287) 0.57/4.62E-02 **Gender/Dx** F-MDD **L:**(2/6) 1/3.20E-02 M-MDD **L:**(16/39) 0.67/4.08E-02				
COL2A1 Collagen Type II Alpha 1 Chain	217404_s_at	(D) DE/4 54%	4	NS		**Gender** Males **C:**(95/426) 0.56/3.53E-02 **Gender/Dx** M-PSYCHOSIS **C:**(33/198) 0.63/7.32E-03 M-SZA **C:**(23/97) 0.66/1.08E-02 **L:**(13/55) 0.66/3.73E-02	**Gender/Dx** M-PTSD **C:**(26/31) 1.83/4.38E-03 **L:**(18/20) 2.3/1.08E-02	Aging		18
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	210747_at	(D) DE/2 44%	8	NS			**All** **C:**(239/501) 1.17/1.03E-02 **Gender** Males **C:**(226/454) 1.19/6.06E-03 **Gender/Dx** M-MDD **C:**(42/72) 1.35/3.68E-02	Addiction Stress	Benzodiazepines	18

					M-PSYCHOSIS **C:**(95/201) 1.26/1.33E-02 M-SZA **C:**(53/98) 1.33/2.06E-02			
					All **C:**(102/470) 0.56/2.38E-02 **L:**(58/287) 0.58/2.49E-02 **Gender** Males **C:**(95/426) 0.56/4.18E-02 L:(54/261) 0.59/1.65E-02 **Gender/Dx** F-MDD **C:**(4/11) 0.82/4.45E-02 M-BP **C:**(18/120) 0.67/1.08E-02 M-MDD **L:**(16/39) 0.67/4.08E-02	**Gender/Dx** F-MDD **C:**(4/12) 7/4.47E-02 M-MDD **L:**(25/43) 2.13/7.30E-03	Suicide	18
Hs.554262	210703_at	(I) AP/6 100%	0	NS				
		(D) DE/6 83%	0	1.59E-02/4 Nominal		**All** **C:**(239/501) 1.13/3.18E-02 **Gender** Males	Alcohol Chronic Stress Longevity Suicide SZ	Clozapine Lithium Valproate
PIK3CD Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta	211230_s_at							18

						C:(226/454) 1.14/2.71E-02 **Gender/Dx** M-BP **C:**(53/134) 1.3/2.85E-02 **L:**(34/91) 1.57/2.01E-02 M-MDD **C:**(42/72) 1.65/5.12E-03			
SVEP1 Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	236927_at	(I) DE/2 49%	4	2.17E-02/4 Nominal	**Gender/Dx** F-PTSD **C:**(5/12) 0.8/4.41E-02 M-PTSD **C:**(13/38) 0.67/4.68E-02	**Gender/Dx** F-MDD **C:**(4/11) 0.82/4.41E-02	Addiction SZ	Omega-3 fatty acids	18
TNFRSF11B TNF Receptor Superfamily Member 11b	204932_at	(D) DE/2 37%	4	2.67E-02/4 Nominal	**Gender/Dx** F-BP **C:**(4/21) 0.81/3.00E-02 M-MDD **L:**(13/43) 0.71/1.72E-02		**Gender/Dx** M-MDD **C:**(42/72) 1.42/4.25E-02 **L:**(25/43) 1.59/3.84E-02	Stress PTSD		18
ZNF91 Zinc Finger Protein 91	244259_s_at	(I) AP/6 95%	0	6.37E-01/2 Stepwise		**Gender/Dx** F-MDD **C:**(4/11) 0.93/1.17E-02	**Gender** Females **C:**(13/47) 2.12/1.03E-02 **Gender/Dx** F-BP	Alcohol Circadian abnormalities PTSD		18

CDK6 Cyclin Dependent Kinase 6	224851_at	(I) DE/4 56% (I) AP/2 42%	4	NS	**Gender/Dx** F-BP **C:**(4/21) 0.78/4.44E-02 L:(3/11) 1/7.15E-03	**All** **C:**(102/470) 0.57/1.03E-02 **Gender** Males **C:**(95/426) 0.59/5.57E-03 **Gender/Dx** M-MDD **C:**(26/67) 0.67/9.11E-03	**C:**(2/16) 4.21/4.55E-02 M-BP **C:**(53/134) 1.35/1.26E-02	Alcohol ASD Circadian abnormalities Longevity MDD SZ	17
EDN1 Endothelin 1	1564630_at	(I) AP/4 56%	4	8.69E-02/2 Stepwise			**Gender** Females **C:**(13/47) 1.9/1.48E-02 **Gender/Dx** M-BP **C:**(53/134) 1.27/2.37E-02		16
(AF090920) PPFIBP2 PPFIA Binding Protein 2	234739_at	(I) AP/6 94%	0	NS	**Gender** Female **C:**(16/65) 0.68/1.42E-02 **L:**(10/36) 0.69/3.87E-02 **Gender/Dx** F-PTSD **C:**(5/12) 0.8/4.41E-02		**Gender/Dx** M-PSYCHOSIS **C:**(95/201) 1.19/3.77E-02 M-SZ **C:**(42/103) 1.22/4.66E-02		16

DCAF12 DDB1 And CUL4 Associated Factor 12	224789_at	(D) DE/6 86%	2	NS	**Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02	**Gender/Dx** M-BP **C:**(53/134) 1.61/4.42E-03	Cocaine Suicide	Omega-3 fatty acids Clozapine	16
DNAJC18 DnaJ Heat Shock Protein Family (Hsp40) Member C18	227166_at	(I) DE/6 94%	0	NS	**Gender** Female **L:**(10/36) 0.78/4.97E-03 **Gender/Dx** F-SZA L:(3/8) 0.93/2.63E-02 F-BP L:(3/11) 0.88/3.31E-02 F-PSYCHOSIS **L:**(3/8) 0.93/2.63E-02 F-PTSD **L:**(3/6) 1/2.48E-02 **Gender/Dx** F-MDD **C:**(4/11) 0.93/1.17E-02	BP		16
HLA-DRB1 Major Histocompatibility Complex, Class II, DR Beta 1	208306_x_at	(I) AP/4 52%	4	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.91/3.39E-02 M-MDD **L:**(13/43) 0.66/4.79E-02 M-SZ L:(7/39) **Gender/Dx** M-SZA **C:**(23/97) 0.62/4.69E-02		Stress PTSD	Antipsychotics	16

					0.71/4.27E-02					
SEPT7P2 Septin 7 Pseudogene 2	1569973_at	**(I)** **DE/6** **100%** (I) AP/2 39%	0	NS	**Gender** Females **C:**(16/65) 0.65/3.27E-02 **Gender/Dx** F-PTSD **C:**(5/12) 0.97/3.69E-03 M-SZ **C:**(11/64) 0.77/2.83E-03		**Gender/Dx** M-MDD **C:**(42/72) 1.45/1.37E-02 **L:**(25/43) 2.25/**5.24E-04**** M-PTSD **C:**(26/31) 2.38/**7.38E-04**** **L:**(18/20) 3.59/1.77E-03	Suicide		16
VEGFA Vascular Endothelial Growth Factor A	212171_x_at	(I) AP/4 65%	4	NS	**Gender/Dx** M-PSYCHOSIS **C:**(19/96) 0.66/1.78E-02 M-SZA **C:**(8/32) 0.7/4.48E-02		**Gender/Dx** M-MDD **C:**(42/72) 1.33/4.83E-02	BP MDD Stress SZ	Lithium Valproate Olanzapine	16
WNK1 WNK Lysine Deficient Protein Kinase 1	1555068_at	(D) DE/6 92%	2	NS	**Gender/Dx** M-MDD **L:**(13/43) 0.77/2.75E-03		**Gender/Dx** M-BP **C:**(53/134) 1.41/3.18E-02	Alcohol Depression Suicide Methamphetamine Stress	Omega-3 Fatty acids SSRI	16
(AF087971) **PBRM1** Polybromo 1	1561067_at	(I) AP/6 90%	0	NS		**All** **C:**(102/470) 0.56/3.71E-02 **Gender** Males **C:**(95/426)		BP Hallucinations Longevity MDD Methamphetamine Mood		14

					0.56/2.87E-02 **Gender/Dx** M-BP **C:**(18/120) 0.63/3.95E-02 M-PSYCHOSIS **C:**(33/198) 0.63/8.63E-03 M-SZA **C:**(23/97) 0.66/1.26E-02		Psychosis Stress Suicide		
(Hs.609761) **SFPQ** Splicing Factor Proline And Glutamine Rich	244331_at	(D) DE/6 98%	0	NS	**Gender/Dx** M-SZ **C:**(11/64) 0.68/3.28E-02 **L:**(7/39) 0.75/2.21E-02		**Gender/Dx** M-MDD **C:**(42/72) 1.68/7.35E-03	Alcohol BP MDD Stress Suicide	Omega-3 fatty acids Clozapine Antidepressants Antipsychotics	14
(Hs.659426) **PHC3** Polyhomeotic Homolog 3	240599_x_at	(D) DE/6 92%	0	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.91/3.39E-02		**Gender/Dx** M-MDD **C:**(42/72) 1.48/1.83E-02	Suicide		14
CCDC85C Coiled-Coil Domain Containing 85C	219018_s_at	(D) DE/6 94%	2	NS	**Gender** Female **L:**(10/36) 0.7/3.31E-02 **Gender/Dx** F-BP **C:**(4/21) 0.79/3.66E-02 **L:**(3/11) 0.92/2.06E-02			Suicide		14

GSPT1 G1 To S Phase Transition 1	215438_x_at	(D) DE/6 94%	0	NS	F-PTSD **L:**(3/6) 1/2.48E-02		**Gender/Dx** M-BP **C:**(53/134) 1.58/4.92E-03	BP Suicide MDD	Valproate	14
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211654_x_at	(I) DE/2 40%	8	NS	**Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02 **Gender/Dx** M-PSYCHOSIS **L:**(10/56) 0.73/1.23E-02 M-SZ **L:**(7/39) 0.81/5.78E-03			Alcohol BP Depression Longevity PTSD Stress Suicide SZ	Antipsychotics	14
LOXL2 Lysyl Oxidase Like 2	228808_s_at	(D) DE/4 59%	4	NS	**Gender** Females **C:**(16/65) 0.66/3.05E-02 **Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02			BP Suicide		14
MBNL3 Muscleblind Like Splicing Regulator 3	219814_at	(D) DE/6 92%	0	NS	**Gender/Dx** M-MDD **L:**(13/43) 0.71/1.51E-02		**Gender/Dx** M-BP **C:**(53/134) 1.43/8.16E-03	Psychosis Hallucination		14
PTN Pleiotrophin	211737_x_at	(D) DE/6 92%	0	NS			**All** **C:**(239/501) 1.16/1.17E-02 **Gender** Males	SZ Stress Suicide	Omega-3 fatty acids Risperidone	14

RALGAPA2 Ral GTPase Activating Protein Catalytic Alpha Subunit 2	231826_at	(D) DE/6 97%	0	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.94/2.46E-02		**C:**(226/454) 1.2/4.66E-03 **Gender/Dx** M-PSYCHOSIS **C:**(95/201) 1.24/1.98E-02 M-SZA **C:**(53/98) 1.35/1.28E-02	BP		14
YBX3 Y-Box Binding Protein 3	201160_s_at	(D) DE/6 94%	0	NS	**Gender/Dx** F-MDD **C:**(2/18) 0.97/1.75E-02		**Gender/Dx** M-BP **C:**(53/134) 1.39/1.23E-02	BP Suicide SZ	Mianserin	14
ZNF441 Zinc Finger Protein 441	1553193_at	(I) AP/6 95% (I) DE/2 35%	0	NS		**Gender/Dx** M-SZA **L:**(13/55) 0.67/3.13E-02	**Gender/Dx** M-MDD L:(25/43) 1.72/1.92E-02			14
CCND1 Cyclin D1	208712_at	(D) DE/4 57%	4	NS			**Gender/Dx** M-BP **C:**(53/134) 1.33/4.53E-02	Addiction MDD Stress Hallucinogens		12
CDK6 Cyclin Dependent Kinase 6	224847_at	(I) DE/4 63%	4	NS			**Gender/Dx** M-PTSD **L:**(18/20) 2.09/1.75E-02	Alcohol ASD Circadian abnormalities Longevity MDD SZ		12
COMT	213981_at	(D)	4	NS	**Gender/Dx**			ADHD	Clozapine	12

Catechol-O-Methyltransferase		DE/4 54%			M-MDD L:(13/43) 0.71/1.41E-02		Aggression Alcohol Anxiety BP Chronic Stress MDD OCD Panic Disorder Psychosis PTSD Suicide SZ	Morphine Mood Stabilizers	
HTR2A 5-Hydroxytryptamine Receptor 2A	211616_s_at	(D) DE/4 52%	4	NS	**Gender/Dx** M-BP L:(16/81) 0.65/2.89E-02		Addictions Aging Alcohol Anxiety BP Depression MDD Mood Disorders NOS OCD Panic Disorder PTSD Stress Suicide SZ		12
NF1 Neurofibromin 1	212676_at	(I) DE/4 59%	4	NS	**Gender/Dx** F-BP L:(3/11) 0.92/2.06E-02		Addiction BP PTSD	Fluoxetine SSRI	12

SHMT1 Serine Hydroxymethyltransferase 1	217304_at	(D) DE/2 43%	6	NS		**Gender/Dx** F-PTSD **C:**(2/8) 1/2.28E-02 M-SZA **L:**(13/55) 0.7/1.54E-02		Suicide	Clozapine	12
TSPO Translocator Protein	202096_s_at	(I) DE/2 38%	6	NS	**Gender/Dx** M-SZ **C:**(11/64) 0.72/1.06E-02			SZ		12
DENND1B DENN Domain Containing 1B	1557309_at	(I) DE/6 90%; (I) AP/2 40%	0	NS	**Gender/Dx** M-SZA **L:**(3/17) 0.83/3.89E-02				Omega-3	10
MCRS1 Microspherule Protein 1	202556_s_at	(I) DE/6 90%	0	NS	**Gender/Dx** M-MDD **L:**(13/43) 0.75/5.16E-03			MDD		10
OSBP2 Oxysterol Binding Protein 2	1569617_at	(D) DE/6 94%	0	NS	**Gender/Dx** F-MDD **C:**(2/18) 1/1.23E-02			Cocaine Suicide SZ		10
FAM134B Family With Sequence Similarity 134 Member B	218510_x_at	(I) DE/4 51%; (I) AP/2 34%	4	NS				Antisocial Personality Suicide	Omega-3 Fatty acids	8
ZNF429 Zinc Finger Protein 429	1561270_at	(D) DE/2 37%	6	NS						8

(Hs.677263) SMURF2 SMAD Specific E3 Ubiquitin Protein Ligase 2	216444_at	(D) AP/6 100% (D) DE/4 71%	0	NS				Aging Suicide Stress		6

DE- differential expression, AP-Absent/Present. NS- Non-stepwise in validation. For Predictions, C-cross-sectional (using levels from one visit), L-longitudinal (using levels and slopes from multiple visits). In All, by Gender, and personalized by Gender and Diagnosis (Gender/Dx).M-males, F-Females. MDD-depression, BP-bipolar, SZ-schizophrenia, SZA-schizoaffective, PSYCHOSIS- schizophrenia and schizoaffective combined, PTSD-post-traumatic stress disorder. Bold and **-significant after Bonferroni correction for the number of biomarkers tested (65). For Steps 2, 5 and 6, see Supplementary Information tables for citations for the evidence.

Table 2. Therapeutics. New Drug Discovery/Repurposing.

A. CMAP Top Biomarkers (n=65 probesets; 19 decreased, 14 increased are present in HG-U133A array used by CMAP)			
rank	CMAP name	score	Description
1	**SC-560**	-1	SC-560 is an NSAID, member of the diaryl heterocycle class of cyclooxygenase (COX) inhibitors which includes celecoxib (Celebrex™) and rofecoxib (Vioxx™). However, unlike these selective COX-2 inhibitors, SC-560 is a selective inhibitor of COX-1.
2	*pyridoxine*	-0.997	Pyridoxine is the 4-methanol form of vitamin B6 and is converted to pyridoxal 5-phosphate in the body. Pyridoxal 5-phosphate is a coenzyme for synthesis of amino acids, neurotransmitters (serotonin, norepinephrine), sphingolipids, aminolevulinic acid.
3	methylergometrine	-0.975	Methylergometrine is a synthetic analogue of ergonovine, a psychedelic alkaloid found in ergot, and many species of morning glory. It is chemically similar to LSD, ergine, ergometrine, and lysergic acid. Due to its oxytocic properties, it has a medical use in obstetrics.
4	LY-294002	-0.923	LY-294002 is a potent, cell permeable inhibitor of phosphatidylinositol 3-kinase (PI3K) that acts on the ATP binding site of the enzyme. The PI3K pathway has a role in inhibiting apoptosis in cancer. PI3K is also known to regulate TLR-mediated inflammatory responses.
5	haloperidol	-0.917	Widely used typical anti-psychotic medication
6	cytisine	-0.909	Like varenicline, cytisine is a partial agonist of nicotinic acetylcholine receptors (nAChRs), with an affinity for the α4β2 receptor subtype, and a half-life of 4.8 hours.
7	*cyanocobalamin*	-0.902	Cyanocobalamin is a form of vitamin B12. Vitamin B12 is important for growth, cell reproduction, blood formation, and protein and tissue synthesis.
8	*apigenin*	-0.899	Apigenin (4′,5,7-trihydroxyflavone), found in many plants such as chamomile, is a natural product belonging to the flavone class. Apigenin acts as a monoamine transporter activator, and is a weak ligand for central benzodiazepine receptors in vitro and exerts anxiolytic and slight sedative effects in an animal model. It has also effects on adenosine receptors and is an acute antagonist at the NMDA receptors (IC50 = 10 μM). In addition, like various other flavonoids, apigenin has been found to possess nanomolar affinity for the opioid receptors, acting as a non-selective antagonist of all three opioid receptors.
9	***beta-escin***	-0.892	Escin, a natural mixture of triterpenoid saponins isolated from horse chestnut (*Aesculus hippocastanum*) seeds, is used and studied as a vasoprotective anti-inflammatory, anti-edematous and anti-nociceptive agent.
13	**amoxapine**	-0.875	Amoxapine is a tricyclic antidepressant of the dibenzoxazepine class. This drug is used to treat symptoms of depression and neuropathic pain.

B. L1000CDS2 Top Biomarkers (n=60 unique genes ; 26 increased and 34 decreased).			
Rank	**Score**	**Drug**	**Description**

1	0.1458	Quinethazone	Thiazide diuretic
2	0.1458	***(-)-Gallocatechin gallate***	Related to the green tea compound EGCG and possible therapeutic molecule for NP treatment due to its anti-inflammatory and antioxidant properties. Interestingly, it has been shown that EGCG reduced bone cancer pain.
3	0.125	***EICOSATRIENOIC ACID (20:3 n-3)***	Omega-3 fatty acid
4	0.125	LFM-A13	Tyrosine kinase inhibitor with anti-inflamatory properties
5	0.125	Picrotoxinin	GABA and glycine receptors inhibitor
6	0.125	INDAPAMIDE	Thiazide-like diuretic
7	0.125	BRD-K15318909	
8	0.125	BRD-K53011428	
9	0.125	BRD-K35100517	
10	0.125	MLS-0454435.0001	
11	0.125	NCGC00181213-02	
12	0.125	ST003833	
13	0.125	STOCK2S-84516	
14	0.125	MLS-0390932.0001	
15	0.125	BRD-K98143437	
16	0.125	BRD-A00993607	
17	0.125	BRD-K68103045	
18	0.125	BRD-K90700939	
19	0.125	triamterene	potassium-sparing diuretic used in combination with thiazide diuretics for the treatment of hypertension and edema.
20	0.1042	PSEUDOEPHEDRINE HYDROCHLORIDE	sympathomimetic drug
21	0.1042	***DOCOSAHEXAENOIC ACID (22:6 n-3)***	Omega-3 fatty acid with antihyperalgesic effect in neuropathic pain
22	0.1042	*Evoxine*	Plant alkaloid with hypnotic and sedative effects.
23	0.1042	Gavestinel	NMDA receptor antagonist
24	0.1042	Mometasone furoate	Corticosteroid
25	0.1042	ZM 241385	denosine A2A receptor antagonist

A. Connectivity Map (CMAP) analysis- drugs that have opposite gene expression profile effects to pain biomarkers signatures. Out of 65 probesets, 14 of the 29 increased, and 19 of the 36 decreased were present in HG-U133A array used by Connectivity Map. A score of -1 indicates the perfect opposite match, i.e., the best potential therapeutic for Pain. B. NIH LINCS analysis using the L1000CDS2 (LINCS L1000 Characteristic Direction Signature Search Engine) tool. Query for signature is done using gene symbols and direction of change. Shown are compounds mimicking direction of change in high memory. A higher score indicates a better match. Bold-drugs known to treat pain, which thus serve as a de facto positive control for the Example. Italic- natural compounds.

Table 3. Demographics. MDD-depression, BP-bipolar, SZ-schizophrenia, SZA-schizoaffective, PSYCHOSIS- schizophrenia and schizoaffective combined, PTSD-post-traumatic stress disorder.

Cohorts	Number of subjects	Gender	Diagnosis	Ethnicity	Age at time of visit Mean (SD)	T-test for age
Discovery						
Discovery Cohort (Longitudinal Within-Subject Changes in Pain Scale) Low Pain 0-2 to High Pain 6-10	28 (with 79 visits)	Male = 19 Female = 9	BP = 9 MDD= 3 SZA= 6 SZ= 3 PTSD= 5 PSYCH= 2	EA= 17 AA= 10 Mixed = 1	52 (7.94)	
Validation						
Independent Validation Cohort (Clinical Severe Pain Diagnosis SF36 sum of scores on questions 21 and 22 ≥10 Pain Scale ≥6)	23 (30 visits)	Male = 13 Female = 10	MDD=8 BP=6 SZ=2 SZA=2 PTSD=2 MOOD=3	EA= 17 AA= 6	51.9 (7.1)	
Testing						
Independent Testing Cohort For Predicting State (High Pain State Pain Scale ≥6 at Time of Assessment)	162 (411 visits)	Male = 134 Female = 28	BP=52 MDD=39 SZA=19 SZ=26 PTSD=20 MOOD=4 PSYCH=2	EA= 112 AA= 48 Hispanic=2	50.3 (8.97) Others 50.12 High Pain 50.50	High Pain (n=101) Vs. Others (n=310) **0.824**
Independent Testing Cohort For Predicting Trait (Future ED visits for Pain in the First Year Following Assessment)	181 (470 visits)	Male = 163 Female = 18	BP = 46 MDD= 33 SZA= 45 SZ= 38 PTSD= 13 MOOD= 4 PSYCH= 2	EA= 117 AA= 62 Hispanic = 2	52.45 (6.13) Others 52.61 ED visits for Pain 51.87	ED visits for Pain (n=102) vs. Others (n=368) **0.237**
Independent Testing Cohort For Predicting Trait (Future ED visits for Pain in All Years Following Assessment)	189 (501 visits)	Male = 170 Female = 19	BP = 49 MDD= 34 SZA= 45 SZ= 40 PTSD=15 MOOD= 4 PSYCH= 2	EA= 124 AA= 62 Hispanic=3	51.79 (6.75) Others 51.58 ED visits for Pain 52.02	ED visits for Pain (n=239) vs. Others (n=262) **0.4720**

Table 4. Top Biomarkers for Pain

Gene Symbol/Gene Name Name	**Probeset**	**Discovery** (Change) Method/Score	Prior Human Genetic Evidence	Prior Human Nervous Tissue Evidence	Prior Human Peripheral Evidence	Prior Non-human Genetic Evidence	Prior Non-human Nervous Tissue Evidence	Prior Non-human Peripheral Evidence	**Prioritization** Total CFG Score For Pain	**Validation** Anova p-value
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	212998_x_at	(I) DE/4 51%		(D) DRG Neurological Pain [1]	(D)Blood Neurological Pain [2]		(I) Spinal Cord Neuropathic Pain [3]		12	NS
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211656_x_at	(I) DE/4 59%		(D) DRG Neurological Pain [1]	(D) Blood Neurological Pain [2]		(I) Spinal Cord Neuropathic Pain [3]		12	NS
CALCA Calcitonin Related Polypeptide Alpha	210727_at	(D) DE/4 54%	Analgesia[4] Migraine [5]		(D) Vertebral disc, Neurological Pain [6] (D) Blood Neuropathic Pain[7] (I) Migraine/Headache [8]		(I) DRG Pain [9] (I) Neurological Pain [10] (I) Dorsal Horn Neurological Pain [11]	(I) blood Acute Pain [12]	11	NS
CCDC144B Coiled-Coil Domain Containing 144B (Pseudogene)	1557366_at	(D) DE/4 56%		(I) Neurological Pain [1]			(D) NAC Neuropathic Pain [13]		10	NS
CNTN1 Contactin 1	1554784_at	(D) DE/4 52%		(D) DRG Neuropathy[14]	(D) CSF[15]				10	NS
GNG7 G Protein Subunit Gamma 7	1566643_a_at	(D) DE/4 59%		(I) sural nerve Diabetic Neuropathy [16]	(I) vertebral disc Neurological				10	6.81E-02 Stepwise

					Pain [6]				
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	210747_at	(D) DE/2 44%		(D) DRG Neurological Pain [1]	(D) Whole blood Neurological Pain [2]		(I) Spinal Cord Neuropathic Pain [3]	10	NS
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211654_x_at	(I) DE/2 40%		(D) DRG Neurological Pain [1]	(D) Whole blood Neurological, Pain [2]		(I) Spinal Cord Neuropathic Pain [3]	10	NS
ASTN2 Astrotactin 2	1554816_at	(I) DE/6 83%	Chronic Migraine [17, 18, 19, 20]					8	1.71E-01 Stepwise
CASP6 Caspase 6	209790_s_at	(I) DE/4 51%			(I) vertebral disc Neurological [6]		DRG Neuropathic pain [21]	8	NS
CCDC85C Coiled-Coil Domain Containing 85C	219018_s_at	(D) DE/6 94%					(I) PAG Neuropathic Pain [13]	8	NS
CCND1 Cyclin D1	208712_at	(D) DE/4 57%			(D) Serum Chronic Pain [22]		(I) (DRG) Neurological Pain [10]	8	NS
CDK6 Cyclin Dependent Kinase 6	224851_at	(I) DE/4 56% (I) AP/2 42%			(D) Serum Chronic Pain [22]		(I) Neuropathic Pain [23]	8	NS
CDK6 Cyclin Dependent Kinase 6	224847_at	(I) DE/4 63%			(D) Serum Chronic Pain [22]		(I) Neuropathic Pain [23]	8	NS
COL2A1 Collagen Type	225293_at	(D)			(D) Lymphoblast		(I) PAG Neuropathic	8	7.47E-01 Stepwise

XXVII Alpha 1 Chain		DE/4 79%		Migraine [24]		Pain [13]		
COL2A1 Collagen Type II Alpha 1 Chain	217404_s_at	(D) DE/4 54%		(I) vertebral disc Neurological Pain [6]		(I) PAG Chronic Neuropathic Pain [13]	8	NS
COMT Catechol-O-Methyltransferase	216204_at	(D) DE/4 54%	Neurological Pain [25, 26] Chronic Pain MSK [27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37] Pain, Acute, Thermal [38] Treatments [39] Pain MSK [29, 28, 27] Pain [40] Morphine [41]	(D) Blood Chronic Pain, MSK [42]			8	NS
COMT Catechol-O-Methyltransferase	213981_at	(D) DE/4 54%	Neurological Pain [25, 26] Chronic Pain MSK [27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37] Pain, Acute, Thermal [38]	(D) blood Chronic Pain, MSK [42]			8	NS

			Treatments [39] Pain MSK [29, 28, 27] Pain [40] Morphine [41]						
DCAF12 DDB1 And CUL4 Associated Factor 12	224789_at	(D) DE/6 86%			(I) Whole blood Neurological, Pain [2]			8	NS
EDN1 Endothelin 1	1564630_at	(I) AP/4 56%	Fibromyalgia [43]		(I) Blister fluid Chronic Pain [44]			8	8.69E-02 Stepwise
FAM134B Family With Sequence Similarity 134 Member B	218510_x_at	(I) DE/4 51%; (I) AP/2 34%	Chronic, Neuropathic Pain [45]		(I) vertebral disc Neurological Pain [6]			8	NS
GBP1 Guanylate Binding Protein 1	231578_at	(I) DE/2 37%	Fibromyalgia [46]	(D) Neurological Pain [1]				8	3.26E-01 Stepwise
HLA-DRB1 Major Histocompatibility Complex, Class II, DR Beta 1	208306_x_at	(I) AP/4 52%	Migraine[47]		(I) Whole blood Neurological Pain [2]			8	NS
HTR2A 5-Hydroxytryptamine Receptor 2A	211616_s_at	(D) DE/4 52%	Neurological, Pain [48] Chronic, MSK [31, 49, 50] Fibromyalgia [51, 52],		(D) whole blood, Neuropathic [7]			8	NS

			Pain, Acute, disease/lesion [54] Pain [40, 55]						
LOXL2 Lysyl Oxidase Like 2	228808_s_at	(D) DE/4 59%			(I) vertebral disc Neurological Pain [6]		(I) PFC Chronic Neuropathic Pain [13]	8	NS
LY9 Lymphocyte Antigen 9	231124_x_at	(I) DE/6 90%					(D) NAC Chronic Neuropathic Pain [13]	8	NS
NF1 Neurofibromin 1	212676_at	(I) DE/4 59%	Migraine [56]		(I) vertebral disc Neurological Pain [6]			8	NS
PNOC Prepronociceptin	205901_at	(I) DE/4 62%			(D) vertebral disc Neurological Pain [6] (I) whole blood Neuropathic Pain [7]		(I) PAG Chronic Neuropathic Pain [13]	8	NS
SHMT1 Serine Hydroxymethyltransferase 1	217304_at	(D) DE/2 43%	Musculoskeletal Pain [57]	(D) Neurological Pain [1]				8	NS
TCF15 Transcription Factor 15 (Basic Helix-Loop-Helix)	207306_at	(D) DE/6 94%					(I) PFC Chronic Neuropathic Pain [13]	8	NS

TOP2A Topoisomerase (DNA) II Alpha	214300_s_at	(D) DE/4 51%		(D) Neurological Pain [1]				8	NS
TSPO Translocator Protein	202096_s_at	(I) DE/2 38%	Neuraxial Pain[58]		(I) vertebral disc Neurological Pain [6]		(I) PAG Neuropathic Pain [13] (I) DRG) Neurological Pain [10]	8	NS
VEGFA Vascular Endothelial Growth Factor A	212171_x_at	(I) AP/4 65%	Neuraxial Pain[59]		(I) Blood Steroid [60] (I) Chronic Pain [61] (I) serum Acute Pain MSK [62]			8	NS
WNK1 WNK Lysine Deficient Protein Kinase 1	1555068_at	(D) DE/6 92%	Chronic Neuropathic Pain [63] Pain [40]					8	NS
ZNF429 Zinc Finger Protein 429	1561270_at	(D) DE/2 37%	Pain MSK [64] Analgesia [65]	(I) Neurological Pain [1]				8	NS
ZYX Zyxin	238016_s_at	(D) DE/4 57%			(I) Whole blood Neurological Pain [2]		(I) PAG Chronic Neuropathic Pain [13]	8	NS
(AF057974) BRMS1 Polybromo 1	1561067_at	(I) AP/6 90%						6	NS
(AF055020) PPP1R2	234739_at	(I) AP/6						6	NS

PPIA Binding Protein 2		94%								
(NS.000788) LRRC75A Leucine Rich Repeat Containing 75A	236913_at	(D) AP/6 97%							6	NS
(NS.000913) PPP1R14B Protein Phosphatase 1 Regulatory Inhibitor Subunit 14B	226138_s_at	(D) DE/6 90%							6	6.28E-02 Stepwise
(NS.000981) SPF45 Splicing Factor Proline And Glutamine Rich	244331_at	(D) DE/6 98%							6	NS
(NS.000985) PRC1 Polyhomeotic Homolog 1	240599_x_at	(D) DE/6 92%							6	NS
(NS.000994) MFAP3 **Microfibril Associated Protein 3**	240949_x_at	(D) DE/6 81%							6	***6.03E-04 Nominal***
(NS.001283) SMURF2 (SMAD Specific E3 Ubiquitin Protein Ligase 2)	216444_at	(D) AP/6 100% (D) DE/4 71%							6	NS
(NS.000420) MTERF Mitochondrial Transcription Termination Factor 1	243125_x_at	(D) DE/6 100%							6	NS
CLSPN Claspin	242150_at	(I) AP/6 95%							6	NS

DENND1B DENN Domain Containing 1B	1557309_at	(I) DE/6 90%; (I) AP/2 40%							6	NS
DNAJC18 DnaJ Heat Shock Protein Family (Hsp40) Member C18	227166_at	(I) DE/6 94%							6	NS
ELAC2 **ElaC Ribonuclease Z 2**	201766_at	(D) DE/4 52%	Fibromyalgia[66]						6	**4.11E-02** ***Nominal***
GSPT1 G1 To S Phase Transition 1	215438_x_at	(D) DE/6 94%							6	NS
HRAS HRas Proto-Oncogene, GTPase	212983_at	(I) DE/6 97%							6	NS
Hs.554262	210703_at	(I) AP/6 100%							6	NS
MBNL3 Muscleblind Like Splicing Regulator 3	219814_at	(D) DE/6 92%							6	NS
MCRS1 Microspherule Protein 1	202556_s_at	(I) DE/6 90%							6	NS
OSBP2 Oxysterol Binding Protein 2	1569617_at	(D) DE/6 94%							6	NS
PIK3CD **Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta**	211230_s_at	(D) DE/6 83%							6	***1.59E-02*** ***Nominal***
PTN Pleiotrophin	211737_x_at	(D) DE/6 92%							6	NS
RAB33A	206039_at	(I)							6	NS

RAB33A, Member RAS Oncogene Family		DE/6 90%								
RALGAPA2 Ral GTPase Activating Protein Catalytic Alpha Subunit 2	231826_at	(D) DE/6 97%							6	NS
SEPTYP2 Septin 7 Pseudogene 2	1569973_at	**(I) DE/6 100%** (I) AP/2 39%							6	NS
SVEP1 **Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1**	236927_at	(I) DE/2 49%	Migraine [56]				(D) NAC Neuropathic Pain [13]		6	***2.17E-02 Nominal***
TNFRSF11B **TNF Receptor Superfamily Member 11b**	204932_at	(D) DE/2 37%	Cancer Pain [67]		(I) vertebral disc Neurological Pain [6] (I) Serum Chronic Pain [68]				6	***2.67E-02 Nominal***
YBX3 Y-Box Binding Protein 3	201160_s_at	(D) DE/6 94%							6	NS
ZNF441 Zinc Finger Protein 441	1553193_at	(I) AP/6 95% (I) DE/2 35%							6	NS
ZNF91 Zinc Finger Protein	244259_s_at	(I) AP/6							6	6.37E-01/2 Stepwise

91		95%								

(n=60 genes, 65 probesets) - evidence for involvement in pain. (I) - increased in expression in Pain, (D)- decreased in expression. DE- differential expression, AP-Absent/Present. DRG- dorsal root ganglia.

Table 5. Top biomarkers for pain - Evidence for involvement in other psychiatric and related disorders.

Gene Symbol/Gene Name Name	Probe set	Discovery (Change) Method/Score	Prioritization Total CFG Score For Pain	Validation Anova p-value	Prior human genetic evidence for other disorders 2pts.	Prior human Brain expression evidence for other disorders 4 pts	Prior human peripheral evidence for other disorders 2pts	Prior Non-human genetic evidence for other disorders 1pt.	Prior Non-human Brain expression evidence for other disorders 2pts.	Prior Non-human peripheral evidence for other disorders 1pt.	External CFG for Other Dx
HTR2A 5-Hydroxytryptamine Receptor 2A	211616_s_at	(D) DE/452%	8	NS	Alcoholism [69] BP [70 71 72 70,73,74] Depression [75-77 78] Mood [79] OCD [80] Addictions [81,82 83 84 85] Suicide [79,86 87-90]	(D) HIP BP [91] (D) HIP SZ, Depression[92] (D) DLPFC BP [92] (D) Temporal Cortex SZ [93] (D) HIP BP, SZ[94]Suicide [95] (D) PFC Aging [96] (D) frontal cortex Suicide [97]	(D) Lymphocyte SZ [103] (D) PBMC SZ [104] (D) Platelets Suicide [105]	Anxiety [106]	(D) PFC SZ [107] (D) Frontal cortex Depression, SZ [108] (D) PFC Hallucinogens [109] (D) AMY PTSD [110]		13

CDK6 Cyclin Dependent Kinase 6	224847_at	(I) DE/4 63%	8	NS	Circadian abnormalities [112] Longevity [113,114] Alcohol [115]	(D) BA46 Suicide [98] (D) Brain BP [99] (I) AMY,Frontopolar cortex Suicide [100] (D) PFC SZ[101] (D) DLFPC Suicide [102]	(I) PFC SZ [116] (I) Brain SZ [117]	(I) lymphoblastoid ASD [118] (I)Blood Female Suicide [119] (I) Blood M-BP Suicide [120]	(I) AMY Depression[111]	10
CDK6 Cyclin Dependent Kinase 6	224851_at	(I) DE/4 56% (I) AP/2 42%	8	NS	Circadian abnormalities [112] Longevity [113,114] Alcohol [115]	(I) PFC SZ [116] (I) Brain SZ [117]	(I) PFC SZ [116] (I) Brain SZ [117]	(I) lymphoblastoid ASD [118] (I)Blood Female Suicide [119] (I) Blood M-BP Suicide [120]	(I) AMY MDD [121]	10
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	212998_at 211656_at	(I) DE/4 (I) DE/4 59%	8	NS NS	Longevity [122,123] SZ [124,125]	(I) Superior temporal cortex (BA 22) SZ [126]	(I) Blood SZ [127] (I) Blood Suicide[128,129] (I) PBMC Stress [130]		(I) CP, NAC (D) AMY Alcoholism [133]	10

WNK1 WNK Lysine Deficient Protein Kinase 1	1555068_at	(D) DE/6 92%	8	NS	Depression [134]		(D) NAC Alcohol [135]	PTSD [131] (I) Leukocytes Depression[132]		10
(AF087971) EEF1G Polybromo 1	1561067_at	(I) AP/6 90%	6	NS	CNV,MDD [137] BP [138-140] [141-143] Mood, Psychosis [144] Depression [139] MDD [139] [140] SZ [141, 145] Longevity[146]	(I) DLPFC BP [139]		(D) Blood Suicide [129, 120]	(I) AMY MDD [121] (I) AMY(male) BP, Stress [136] (I) Brain Stimulants [149]	10
(NM_033308) MFAP3 Microfibril Associated Protein 3	240949_x_at	(D) DE/6 81%	6	***6.03E-04/4 Nominal***	SZ [124]	(D)Superior frontal cortex Alcohol [150]		(D)Blood Suicide [129, 120]	(D) AMY Stress [121]	10
CCND1 Cyclin D1	208712_at	(D) DE/4 57%	8	NS		(D) Frontal motor cortex Alcohol [151] (D) hippocampus Alcohol [152] (D) ACC MDD [153]	Addiction Alcohol [155]	(D) Peripheral blood Stress [154]	(D) Amygdala) Hallucinogens [156] (D) Amygdala Addiction Alcohol [133]	9
CNTN1 Contactin 1	1554784_at	(D) DE/4 52%	10	NS	BP,SZ [157; 158] MDD [134] Suicide [159]	(D) Brain BP [99] (D) HIP BP [160] (D) Forebrain neural progenitor cells		(D) lymphocyte SZ [164] (D) Blood Female Suicide [119]		8

					SZ [161] (D) supragenual (BA24) anterior cingulated cortex SZ [162] (D) anterior PFC SZA [163]				
GBP 1 Guanylate Binding Protein 1	231578_at	(I) DE/237%	8	3.26E-01/2 Stepwise	(I) Hippocampus, amygdala, gyrus cinguli ,pons MDD [165] (I) amygdala SZ [166] (I) left side superior frontal gyrus SZ [167] (I) Brain Suicide [165]	(I) leukocytes PTSD [168]		(I) hippocampal and prefrontal cortex MDD [165]	8
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211654_x_at	(I) DE/240%	8	NS	(I) superior temporal cortex SZ [126]	(I) monocytes Stress [130] (I) PBMC PTSD [131]		(I) Caudate putamen Addiction Alcohol[133]	8
PNOC Prepronociceptin	205901_at	(I) DE/462%	7	NS	(I) DLPFC BP,SZ [170] (I) AMY, cingulate cortex MDD [171] (I) Forebrain neural cells SZ [161]	(I) Fibroblasts SZ [172]	Addictions [169]	(I) NAC Stress [173] (I) Amygdala MDD [111]	8
SSNA1 G1 To S Phase	215438_x_at	(D) DE/694%	6	NS	(D) Brain BP [99]	(D) Blood Suicide [129]		(D) AMY Depression[121]	8

Transition 1							(D) Leukocytes Depression[132]				
(Hs.60976 1) SFPQ Splicing Factor Proline And Glutamine Rich	244331_at	(D) DE/6 98%	6	NS		NAC altered MDD [174] (D) superior frontal cortex Alcohol [150] (D) PFC MDD [175]	(D) Blood Female Suicide [119]		(D) VT Hallucinogens [156] (D) PFC (male) Stress, BP [136] (D) Brain Alcohol Addiction[176]		8
ZNF91 Zinc Finger Protein 91	244259_s_at	(I) AP/6 95%	6	6.37E-01/2 Stepwise	Circadian abnormalities [112]	(I) Temporal cortex Alcoholism [177] (I) DLPFC PTSD [178]	(I) Blood PTSD [179]				8
COMT Catechol-O-Methyltransferase	216204_at 213981_at	(D) DE/4 54% (D) DE/4	8	NS NS	OCD [180 181 182 183-185 186] BP [187 182 188 189, 190 191, 192] Anxiety[193 194 195 196 197 198 199 200 201] SZ [202 203 204 205 206 207 192, 208 209] Aggression [210 211 212] Suicide [213 214 215 216 217] Thermal [218] Stimulants[219] Intellect[220] Mood [209]		(D) Blood Alcoholism [227] (D) Blood SZ [228] (D) Leukocytes SZ [229] (D) PBMC Stress [130] (D) Blood Suicide[119]	SZ[230, 231] Alcoholism [155]	(D) PFC Anxiety, OCD, SZ[232] (D) Brain Anxiety [233] (D) Male HIP, AMY Anxiety [234]		7

VEGFA Vascular Endothelial Growth Factor A	2121 71_x_ at	(I) AP/4 65%	8	NS	ADHD [186] Depression[78] [221-223] PTSD [224] [225] Alcohol[226]	(I) CA3/2 Stratum oriens SZ [235] (I) Prefrontal cortex SZ [236] (I) hippocampus SZ [237]	(I) monocytes Stress [130] (I) plasma MDD [238] (I) plasma BP [239]	MDD [240]		7
[illegible] Leucine Rich Repeat Containing 75A	2369 13_at	(D) AP/6 97%	6	NS		(D) Brain BP [99] (D) DLPFC SZ [241]	(D) Blood Male BP Suicide [120]	Alcohol Addiction[155]		7
CALCA Calcitonin Related Polypeptide Alpha	2107 27_at	(D) DE/4 54%	7	NS		(D) Frontal, motor cortex Alcohol[151]			(D) Medullae Oblongata Anxiety [242]	6
LOXL2 Lysyl Oxidase Like 2	2288 08_s_ at	(D) DE/4 59%	7	NS		(D) anterior PFC BP [163]	(D) Male-BP Suicide[120]			6
HRAS HRas Proto-Oncogene, GTPase	2129 83_at	(I) DE/6 97%	6	NS	BP, SZ [157] Longevity [243]		mRNA Suicide [244]		(I) NAC Alcohol [245]	6
[illegible] MTERF4 Mitochondrial Transcription Terminati	2431 25_x_ at	(D) DE/6	6	NS		(D) DPFC BA 46 PTSD [246]	(D) Blood Universal Suicide [120]			6

on Factor 1											
PIK3CD Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta	2112 30_s_ at	(D) DE/6 83%	6	***1.59E-02/4 Nominal***	Longevity [247] SZ [248]		(D) PBMC Stress [130] (D) Blood Suicide [120] mRNA Suicide [244]		(D) NAC Alcohol [133]		6
PTN Pleiotrophin	2117 37_x_ at	(D) DE/6 92%	6	NS	SZ [145] [249]		mRNA Suicide [244]		(D) HIP Stress[250]		6
YBX3 Y-Box Binding Protein 3	2011 60_s_ at	(D) DE/6 94%	6	NS		(D) DLPFC BP,SZ [170]	(D) Blood Male Suicide [129]				6
NF1 Neurofibromin 1	2126 76_at	(I) DE/4 59%	8	NS		Differentially expressed ACC (BA 24) BP [251]		Addiction Alcohol [155]	(I) VS PTSD [110]		5
SVEP1 Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	2369 27_at	(I) DE/2 49%	6	***2.17E-02/4 Nominal***		(I) Hippocampus SZ [252]		Alcohol [155]			5

SMURF2 SMAD Specific E3 Ubiquitin Protein Ligase 2	216444_at	(D) AP/6 100 % (D) DE/4 71%	6	NS			(D) Blood Suicide [129, 120]	(D) VM PFC Stress [253]	Intervertebral disc Aging [254]	5
ASTN2 Astrotactin 2	1554816_at	(I) DE/6 83%	8	1.71E-01 Stepwise	Stimulants[255] SZ[256, 257, 206] Autism[258] Autism CNV[259] BP[257]		(I) Female Blood Suicide[119]			4
CASP6 Caspase 6	209790_s_at	(I) DE/4 51%	8	NS		(I) Dorsolateral prefrontal cortex BP [260]				4
FAM134B Family With Sequence Similarity 134 Member B	218510_x_at	(I) DE/4 51%; (I) AP/2 34%	8	NS	Antisocial Personality [261]		(I) Male BP SI, Universal SI[120]	(I) VT Hallucinogens [156]		4
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	210747_at	(D) DE/2 44%	8	NS			(D) leukocytes Stress, [262]	(D) Amygdala Addictions, Alcohol [133]		4
ZYX Zyxin	238016_s_at	(D) DE/4 57%	7	NS			(D) Blood MDD [263]	(D) AMY MDD [264]		4
DNAJC18 DnaJ Heat Shock Protein	227166_at	(I) DE/6	6	NS		ACC (BA 24) BP [265]				4

Family (Hsp40) Member C18										
MORN1 Microsphe rule Protein 1	2025 56_s_ at	(I) DE/6	6	NS		(I) Pituitary Depression[266]				4
OSBP2 Oxysterol Binding Protein 2	1569 617_a t	(D) DE/6	6	NS	SZ [267]		(D) Blood Suicide [128, 129] (D) SH-SY5Y cells Cocaine [268]			4
RAB33A RAB33A, Member RAS Oncogene Family	2060 39_at	(I) DE/6	6	NS		(I) Frontal Cortex Alcohol [269] (I) Stress[270] (I)PFC,ACC,MD D[153]				4
TSPO Translocat or Protein	2020 96_s_ at	(I) DE/2 38%	6	NS		(I) Forebrain neural progenitor cells SZ [161]				4

GNG7 G Protein Subunit Gamma 7	1566643_a_at	(D) DE/4 59%	10	6.81E-02/2 Stepwise					(D) NAC Alcohol [271] (D) PFC Hallucinogens [156] (D) PFC (male) BP/Stress [136] (D) AMY MDD [111]		2
COL27A1 Collagen Type XXVII Alpha 1 Chain	225293_at	(D) DE/4 79%	8	7.47E-01/2 Stepwise	Tourette syndrome [272]						2
DCAF12 DDB1 And CUL4 Associated Factor 12	224789_at	(D) DE/6 86%	8	NS			(D) SH-SY5Y cells Cocaine [268] (D) Blood Universal Suicide[120]				2
SHMT1 Serine Hydroxymethyltransferase 1	217304_at	(D) DE/2 43%	8	NS			(D) Blood Suicide [129,120]				2
(Hs.596713)	226138_s_	(D) DE/6	6	6.28E-02					(D) parietal cortex SZ[273]		2

PPP1R14B Protein Phosphatase 1 Regulatory Inhibitor Subunit 14B	at	90%		Stepwise					
CCDC85C Coiled-Coil Domain Containing 85C	219018_s_at	(D) DE/6 94%	6	NS			(D) Male Blood Suicide [129]		2
CLSPN Claspin	242150_at	(I) AP/6 95%	6	NS			(I) Blood Suicide [119, 129]		2
ELAC2 **ElaC Ribonuclease Z 2**	201766_at	(D) DE/4 52%	6	***4.11E-02/4 Nominal***	Autism[274]				2
Hs.554262	210703_at	(I) AP/6	6	NS			(I) Blood Universal Suicide[120]		2
PNISR PNN Interacting Serine-Rich Protein	240599_x_at	(D) DE/6	6	NS			(D) Blood Female Suicide [119]		2
LY9 Lymphocyte Antigen 9	231124_x_at	(I) DE/6 90%	6	NS			(D) Blood Stress[275]		2
MBNL3 Muscleblind Like Splicing Regulator	219814_at	(D) DE/6 92%	6	NS			(D) Blood Hallucinations [147]		2

3										
RALGAPA2 Ral GTPase Activating Protein Catalytic Alpha Subunit 2	231826_at	(D) DE/6 97%	6	NS	BP [70]					2
SEPTIN7P2 Septin 7 Pseudogene 2	156997 3_at	**(I) DE/6 100 %** (I) AP/2 39%	6	NS			(I) Blood Suicide[119]			2
TCF15 Transcription Factor 15 (Basic Helix-Loop-Helix)	207306_at	(D) DE/6 94%	6	NS			(D) Blood Suicide [129, 120]			2
TNFRSF11B **TNF Receptor Superfamily Member 11b**	204932_at	(D) DE/2 37%	4	***2.67E-02/4 Nominal***					(D) Hippocampus Stress[121] (D) PFC Stress [253] (D) HC PTSD [110]	2
HLA-DRB1 Major Histocompatibility Complex, Class II, DR Beta 1	208306_x_at	(I) AP/4 52%		NS			(I) leukocytes Stress [262] (I) Blood PTSD [276]			2
CORO1A	1557	(D)	10	NS						0

B Coiled-Coil Domain Containing 144B (Pseudogene)	366_at	DE/4 56%							0
COL2A1 Collagen Type II Alpha 1 Chain	217404_s_at	(D) DE/4 54%	7	NS					0
[illegible] PPFIA Binding Protein 2	234739_at	(I) AP/6 94%	6	NS					0
DENND1B DENN Domain Containing 1B	1557309_at	(I) DE/6 90%	6	NS					0
ZNF441 Zinc Finger Protein 441	1553193_at	(I) AP/6 95% (I) DE/2 35%	6	NS					0
TOP2A Topoisomerase (DNA) III Alpha	214300_s_at	(D) DE/4 51%	4	NS					0
ZNF429 Zinc Finger Protein 429	1561270_at	(D) DE/2 37%	2	NS					0

In the same direction of expression. (I)- increased in expression in Pain, (D)- decreased in expression. DE- differential expression, AP- Absent/Present.

Table 6. Biological Pathway Analysis:

	DAVID GO Functional Annotation Biological Processes					KEGG Pathways				Ingenuity Pathways (Fold change)		
A.	#	Term	Count	%	P-Value	Term	Count	%	P-Value	Top Canonical Pathways	P-Value	Overlap
60 Pain Genes (n= 60 Genes,65 probesets)	1	regulation of homeostatic process	11	18.6	1.10E-06	Focal adhesion	7	11.9	7.20E-05	Hereditary Breast Cancer Signaling	3.36E-05	3.5 % 5/144
	2	epithelial cell proliferation	8	13.6	9.60E-05	PI3K-Akt signaling pathway	8	13.6	1.60E-04	Ovarian Cancer Signaling	3.36E-05	3.5 % 5/144
	3	T cell receptor signaling pathway	6	10.2	1.70E-04	Non-small cell lung cancer	4	6.8	1.00E-03	Non-Small Cell Lung Cancer Signaling	4.53E-05	5.2 % 4/77
	4	aging	7	11.9	2.30E-04	Pancreatic cancer	4	6.8	1.60E-03	Glioblastoma Multiform Signaling	5.89E-05	3.1 % 5/162
	5	negative regulation of multicellular organismal process	12	20.3	2.50E-04	Glioma	4	6.8	1.60E-03	HER-2 Signaling in Breast Cancer	7.65E-05	4.5 % 4/88

B.	David				Ingenuity Pathways Disease		
	#	Term	Count	%	P-Value	Diseases and Disorders	P-Value	# Molecules
60 Pain Genes (n=60 Genes, 65 probesets)	1	Mood disorders	5	8.5	2.00 E-05	Neurological Disease	2.5 E-05 – 3.26 E-08	30
	2	Head and Neck Cancer	6	10.2	2.10 E-05	Cancer	2.50 E-03 – 9.87 E-08	54
	3	Arthritis, Rheumatoid/Rheumatoid Arthritis	7	11.9	4.40 E-05	Organismal Injury and Abnormalities	2.56 E-03 – 9.87 E-08	55
	4	Autism	9	15.3	4.40 E-05	Reproductive System Disease	1.86 E-03 – 1.79 E-07	37
	5	Glomerulonephritis, IGA	6	10.2	6.30 E-05	Renal and Urological Disease	1.44 E-03 – 1.11 E-06	16

Table 7. Pharmacogenomics. Top list biomarkers in datasets that are targets of existing drugs and are modulated by them in opposite direction.

Gene Symbol/Gene Name Name	Probeset	Discovery (Change) Method/Score	Prioritization Total CFG Score For Pain	Validation Anova p-value	Pain Medications	Omega-3	Antidepressants	Mood Stabilizers	Antipsychotics	Others
CNTN1 Contactin 1	1554784_at	(D) DE/4 52%	10	NS					(I) VT Clozapine [156]	
GNG7 G Protein Subunit Gamma 7	1566643_a_at	(D) DE/4 59%	10	6.81E-02/2 Stepwise		(I)Brain Omega-3 fatty acids[277] (I)AMY(females) Omega-3 fatty[278]				
ASTN2 Astrotactin 2	1554816_at	(I) DE/6 83%	8	1.71E-01 Stepwise					Antipsychotics [279]	
CDK6 Cyclin Dependent Kinase 6	224851_at	(I) DE/4 56% (I) AP/2 42%	8	NS						palbociclib, ribociclib, abemaciclib, letrozole/palbociclib, FLX925, fulvestrant/palbociclib, trilaciclib, G1T38, letrozole/ribociclib, abemaciclib/fulvestrant, alvocidib
CDK6 Cyclin Dependent Kinase 6	224847_at	(I) DE/4 63%	8	NS						

COL27A1 Collagen Type XXVII Alpha 1 Chain	225293_at	(D) DE/4 79%	8	7.47E-01/2 Stepwise					
COMT Catechol-O-Methyltransferase	213981_at; 216204_at	(D) DE/4 54%	8	NS	Morphine [41] Thermal [218]		Mood Stabilizers [281]	(I) VT Clozapine [156]	
DCAF12 DDB1 And CUL4 Associated Factor 12	224789_at	(D) DE/6 86%	8	NS		(I) Lymphocytes (females) Omega-3 fatty acids [278]		(I) Lymphocytes Clozapine [156]	
FAM134B Family With Sequence Similarity 134 Member B	218510_x_at	(I) DE/4 51%; (I) AP/2 34%	8	NS		(D) Lymphocytes (females) Omega-3 fatty acids [278]			
GBP1 Guanylate Binding Protein 1	231578_at	(I) DE/2 37%	8	3.26E-01/2 Stepwise		(D) Blood Omega-3 fatty acids [278]			
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	210747_at	(D) DE/2 44%	8	NS				(I)Blood Benzodiazepines [282]	
HLA-DQB1 Major	211654_x_at	(I) DE/2	8	NS					(D)PFC Antipsyc

Histocompatibility Complex, Class II, DQ Beta 1		40%						hotics [283]	
HLA-DQB1 Major Histocompatibility Complex, Class II, DQ Beta 1	211656_x_at; 212998_x_at	(I) DE/4 59%	8	NS				(D) PFC Antipsychotics [283]	
HLA-DRB1 Major Histocompatibility Complex, Class II, DR Beta 1	208306_x_at	(I) AP/4 52%	8	NS				(D)PFC Antipsychotics [283]	apolizumab
HTR2A 5-Hydroxytryptamine Receptor 2A	211616_s_at	(D) DE/4 52%	8	NS					Hallucinogens
NF1 Neurofibromin 1	212676_at	(I) DE/4 59%	8	NS			(D) cerebral cortex Fluoxetine SSRI [284]		
SHMT2 Serine Hydroxymethyltransferase 1	217304_at	(D) DE/2 43%	8	NS				(I)VT Clozapine [156]	
TOP2A Topoisomerase (DNA) III Alpha	214300_s_at	(D) DE/4 51%	8	NS		(I)Brain Omega-3 fatty acids[277]			

VEGFA Vascular Endothelial Growth Factor A	212171_x_at	(I) AP/4 65%	8	NS			(D) lymphoblastoid cell cultures Lithium, Valproate [285]	(D) HIP and cerebellum Olanzapine [286]	Anti-cancer mABs
WNK1 WNK Lysine Deficient Protein Kinase 1	1555068_at	(D) DE/6 92%	8	NS	(I) Lymphocytes (females) Omega-3 fatty acids[278]	(I) cingulate cortex SSRI (Fluoxetine)[264]			
CALCA Calcitonin Related Polypeptide Alpha	210727_at	(D) DE/4 54%	7	NS	(I) HIP (males) Omega-3 fatty acids[278]		(I) Schneider 2 cells Lithium[287]		
ZYX Zyxin	238016_s_at	(D) DE/4 57%	7	NS				(I) Lymphocytes Clozapine [156]	
LRRC75A Leucine Rich Repeat Containing 75A	236913_at	(D) AP/6 97%	6	NS				(I) HIP Clozapine[156]	
C18orf63 Chromosome 18 Open Reading Frame 63 Protein	226138_s_at	(D) DE/6 90%	6	6.28E-02 Stepwise			(I) Schneider 2 (S2)		

Phosphatase 1 Regulatory Inhibitor Subunit 14B							cells, Lithium [287]		
(PSF) SFPQ Splicing Factor Proline And Glutamine Rich	244331_at	(D) DE/6 98%	6	NS	(I) HIP (males) Mood, Omega-3 fatty acids[278]	(I) basal forebrain TCA[288]		(I) PFC Clozapine[156]	
DENND1B DENN Domain Containing 1B	1557309_at	(I) DE/6 90%; (I) AP/2 40%	6	NS	(D) Brain Omega-3 fatty acids[277]				
G1S1 G1 To S Phase Transition 1	215438_x_at	(D) DE/6 94%	6	NS			(I) CP Valproate [289]		
HRAS HRas Proto-Oncogene, GTPase	212983_at	(I) DE/6 97%	6	NS					ISIS 2503
LY9 Lymphocyte Antigen 9	231124_x_at	(I) DE/6 90%	6	NS	(D) Brain Omega-3 fatty acids [277]				
PIK3CD **Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta**	211230_s_at	(D) DE/6 83%	6	***1.59E-02/4 Nominal***			(I) Lymphoblastoid cells Lithium, Valproate [285]	(I) VT Clozapine[156]	
PTN Pleiotrophin	211737_x_at	(D) DE/6 92%	6	NS	(I) HIP (males) Omega-3 fatty acids [278]				

SVEP1 Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	236927_at	(I) DE/2 49%	6	***2.17E-02/4 Nominal***		(I) fronto-temporo-parietal cortex Antipsychotics(risperidone)[290]	(D)Brain Omega-3 fatty acids[277]	CGS-8216, dexamethasone/olanzapine, fluoxetine/olanzapine, estazolam, clorazepate, eszopiclone, temazepam, zolpidem, chlordiazepoxide, lorazepam, olanzapine, triazolam, flumazenil, clonazepam, flurazepam, midazolam, flunitrazepam, alprazolam, zaleplon, SSR180575, PK 11195
TSPO Translocator Protein	202096_s_at	(I) DE/2 38%	6	NS				

YBX3 Y-Box Binding Protein 3	201160_s_at	(D) DE/6 94%	6	NS			(I) c.elegans mianserin [291]

CLAIMS

What is claimed is:

1. A method for treating pain in a subject in need thereof, the method comprising: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker; and administering a treatment, wherein the treatment reduces the difference between the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker to mitigate pain in the subject.

2. The method of claim 1, wherein the biomarker is selected from the group of biomarkers listed in Table 1 and combinations thereof.

3. The method of claim 1, wherein the biomarker is selected from the group consisting of microfibril associated protein 3 (MFAP3), phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD), Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1 (SVEP1), TNF Receptor Superfamily Member 11b (TNFRSF11B), ElaC Ribonuclease Z 2 (ELAC2), and combinations thereof.

4. The method of claim 1, wherein the treatment is selected from the group listed in Tables 1, 2A, 2B and 7 and combinations thereof.

5. The method of claim 1, wherein the biomarker is decreased in response to the treatment.

6. The method of claim 1, wherein the biomarker is increased in response to the treatment.

7. A method for determining intensity of pain in an subject in need thereof, the method comprising: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; and identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker, wherein the difference in the expression level of the blood biomarkers in the sample obtained from the subject and the reference expression level of the blood biomarkers determines the intensity of pain.

8. The method of claim 7, wherein the biomarker is selected from the group of biomarkers listed in Table 1 and combinations thereof.

9. The method of claim 7, wherein the biomarker is selected from the group consisting of microfibril associated protein 3 (MFAP3), phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD), Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1 (SVEP1), TNF Receptor Superfamily Member 11b (TNFRSF11B), ElaC Ribonuclease Z 2 (ELAC2), and combinations thereof.

10. The method of claim 7, wherein the expression level of the blood biomarker in the sample obtained from the subject is increased as compared to the reference expression level of the biomarker.

11. The method of claim 7, wherein the expression level of the blood biomarker in the sample obtained from the subject is decreased as compared to the reference expression level of the biomarker.

12. A method for predicting a future medical care facility visit for pain in a subject in need thereof, the method comprising: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker, wherein the difference in the expression level of the blood biomarkers in the sample obtained from the subject and the reference expression level of the blood biomarkers determines the likelihood of future medical facility visits for pain.

13. The method of claim 12, wherein the biomarker is selected from the group of biomarkers listed in Table 1 and combinations thereof.

14. The method of claim 12, wherein the expression level of the blood biomarker in the sample obtained from the subject is increased as compared to the reference expression level of the biomarker.

15. The method of claim 12, wherein the expression level of the blood biomarker in the sample obtained from the subject is decreased as compared to the reference expression level of the biomarker.

16. A method for mitigating pain in a subject in need thereof, the method comprising: obtaining an expression level of a blood biomarker in a sample obtained from the subject; obtaining a reference expression level of the blood biomarker; identifying a difference in the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker; and administering a treatment, wherein the treatment reduces the difference between the expression level of the blood biomarker in the sample and the reference expression level of the blood biomarker to mitigate pain in the subject.

17. The method of claim 16, wherein the treatment is selected from the group listed in Tables 1, 2A, 2B and 7, and combinations thereof.

18. The method of claim 16, wherein the treatment is selected from the group consisting of SC-560, pyridoxine, methylergometrine, LY-294002, haloperidol, cytisine, cyanocobalamin, betaescin, amoxapine, apigenin and combinations thereof.

19. The method of claim 16, wherein the treatment is selected from the group listed in Table 2B, and combinations thereof.

20. A method for identifying a blood biomarker for pain, the method comprising: obtaining a first biological sample from a subject and administering a first pain intensity test to the subject; obtaining a second biological sample from the subject and administering a second pain intensity test to the subject; identifying a first cohort of subjects by identifying subjects having a change from low pain intensity to high pain intensity as determined by a difference between the first pain intensity test and the second pain intensity test; identifying candidate biomarkers in the first cohort by identifying biomarkers having a change in expression between the first biological sample and the second biological sample.

21. The method of claim 20 further comprising prioritizing the candidate biomarkers by identifying candidate biomarkers known to be associated with pain.

22. The method of claim 20, wherein the pain intensity test is selected from the group consisting of Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), and combinations thereof.

FIG. 1A

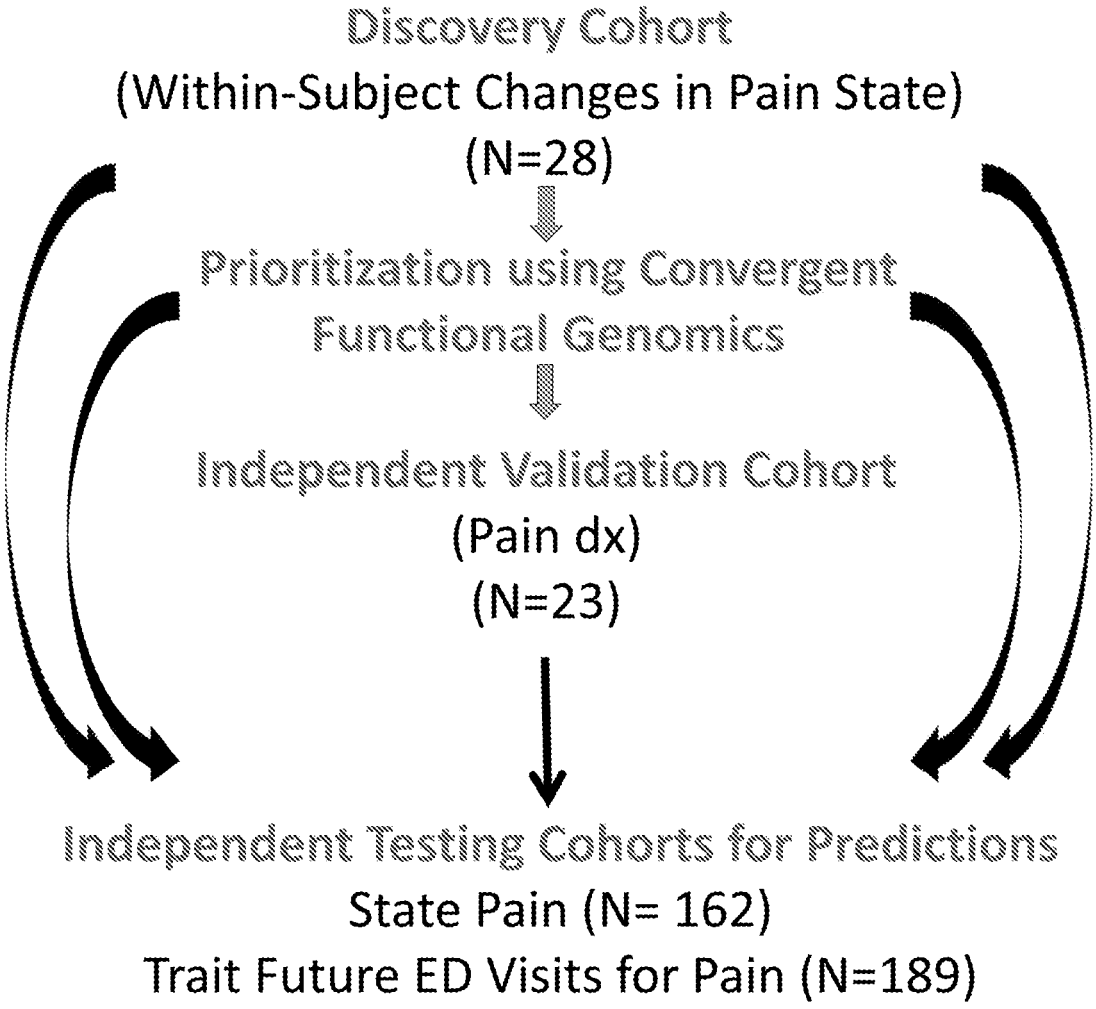
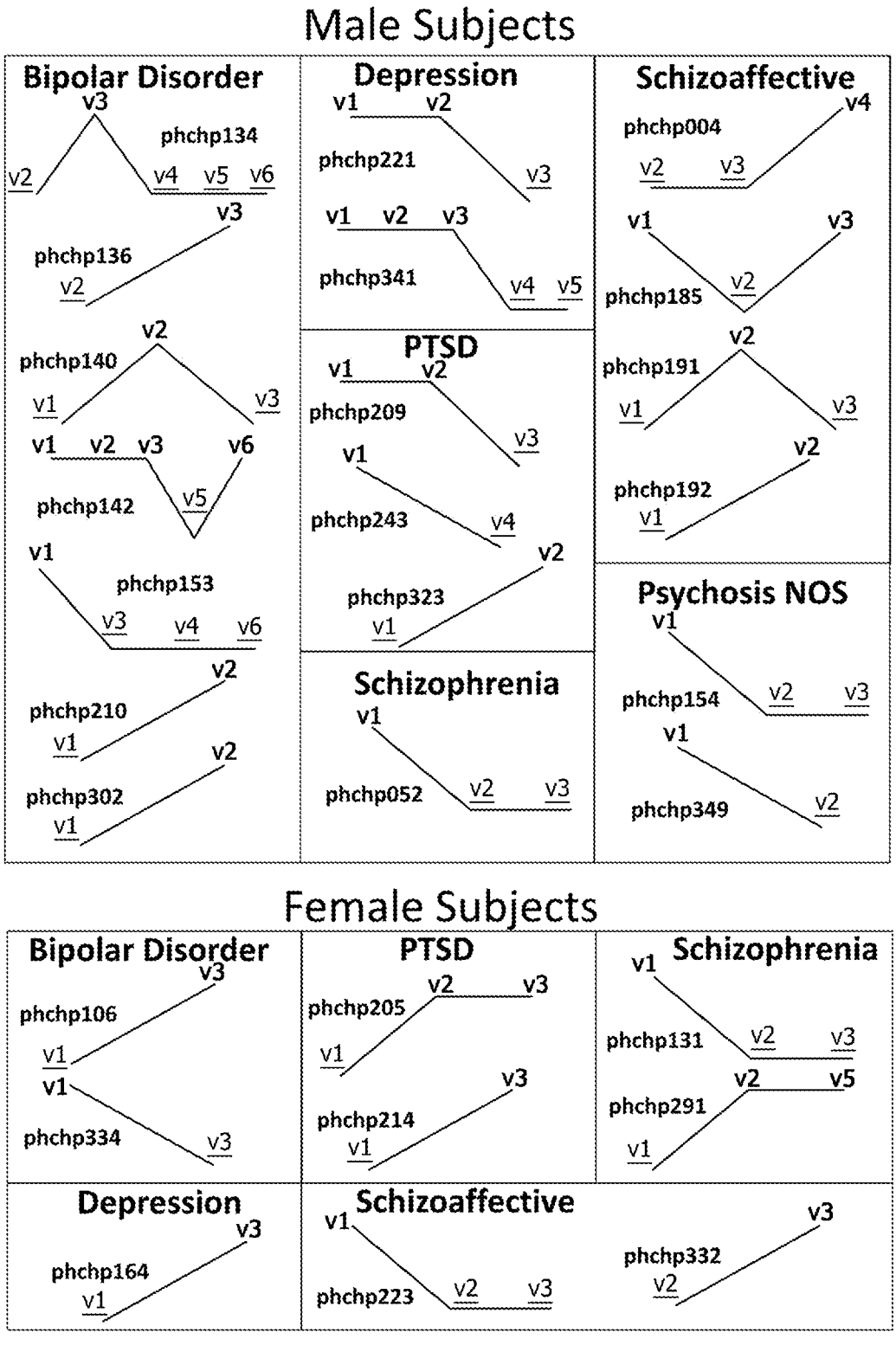

Discovery Cohort
(Within-Subject Changes in Pain State)
(N=28)
Prioritization using Convergent Functional Genomics
Independent Validation Cohort
(Pain dx)
(N=23)
Independent Testing Cohorts for Predictions
State Pain (N= 162)
Trait Future ED Visits for Pain (N=189)

FIG. 1B

Discovery Cohort:

19 male and 9 female psychiatric participants who have at least one switch between a Low Pain state visit and a **High Pain** state visit.



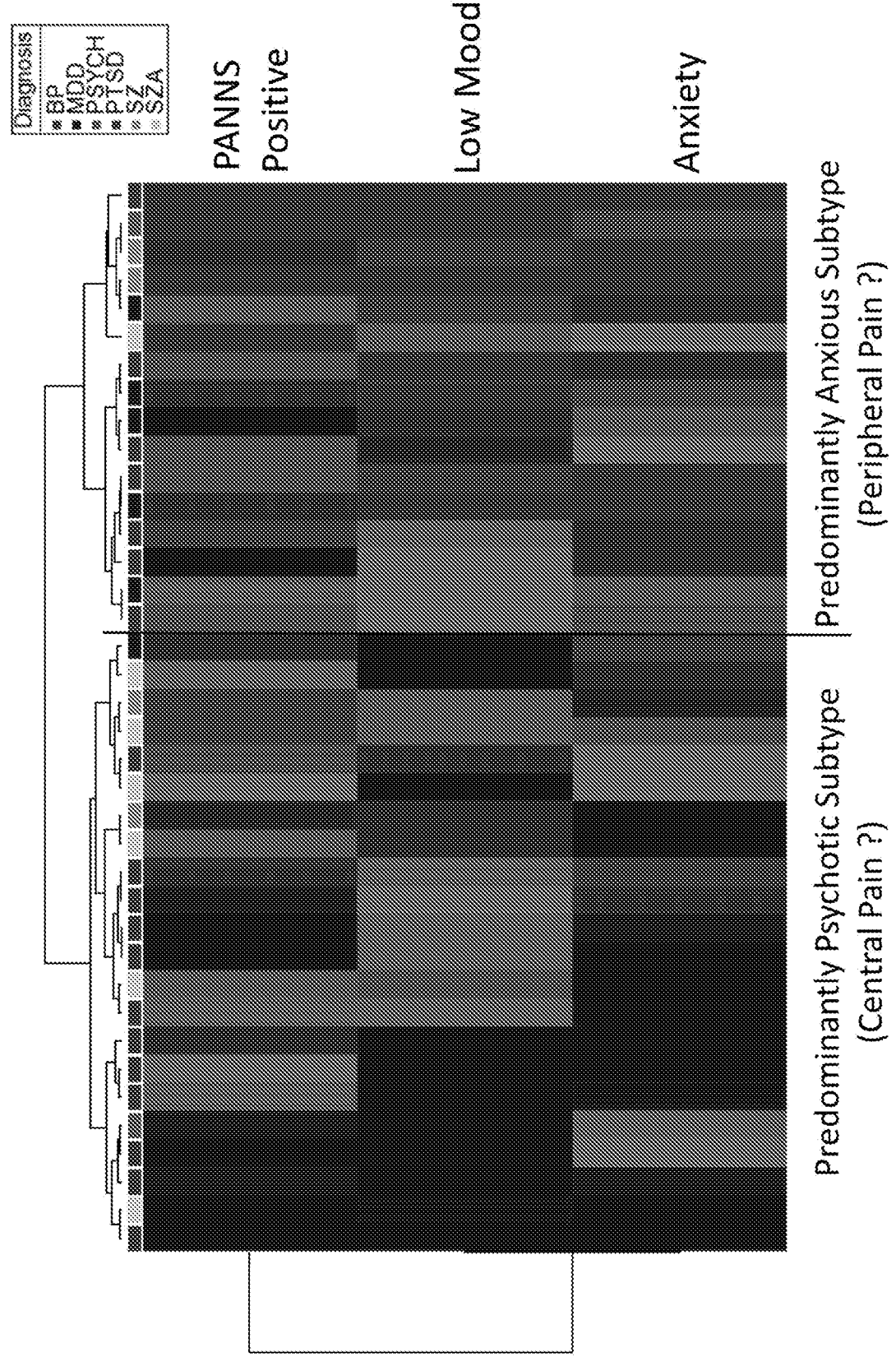
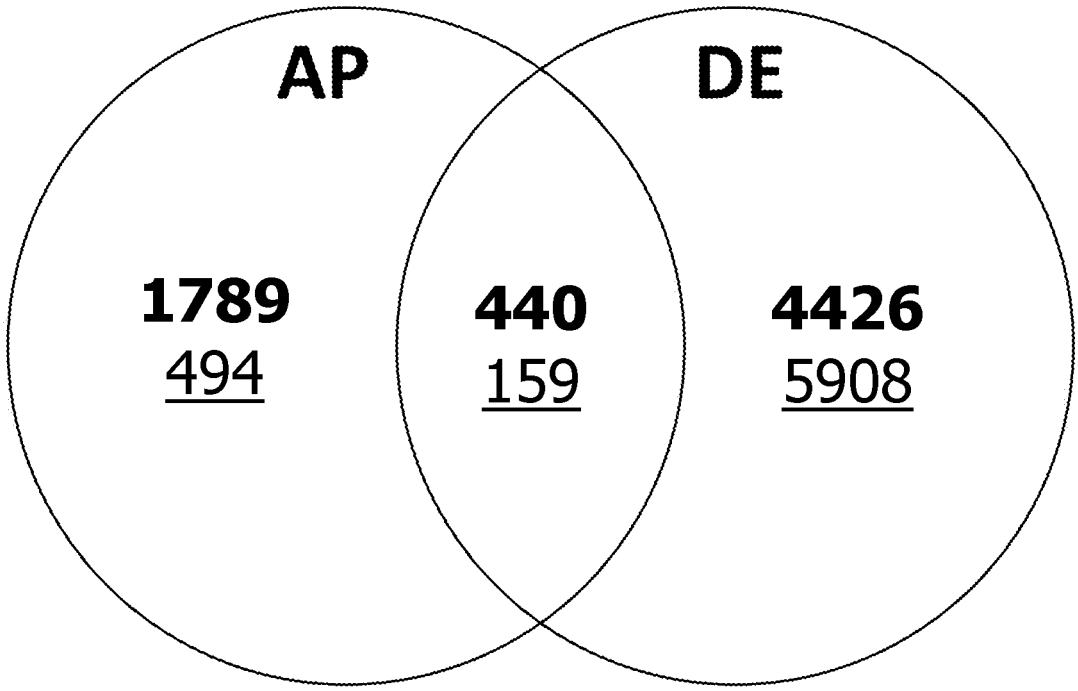

Diagnosis
BP
MDD
PSYCH
PTSD
SZ
SZA
PANNS Positive
Low Mood
Anxiety
Predominantly Anxious Subtype (Peripheral Pain ?)
Predominantly Psychotic Subtype (Central Pain ?)

FIG. 1C

FIG. 1D



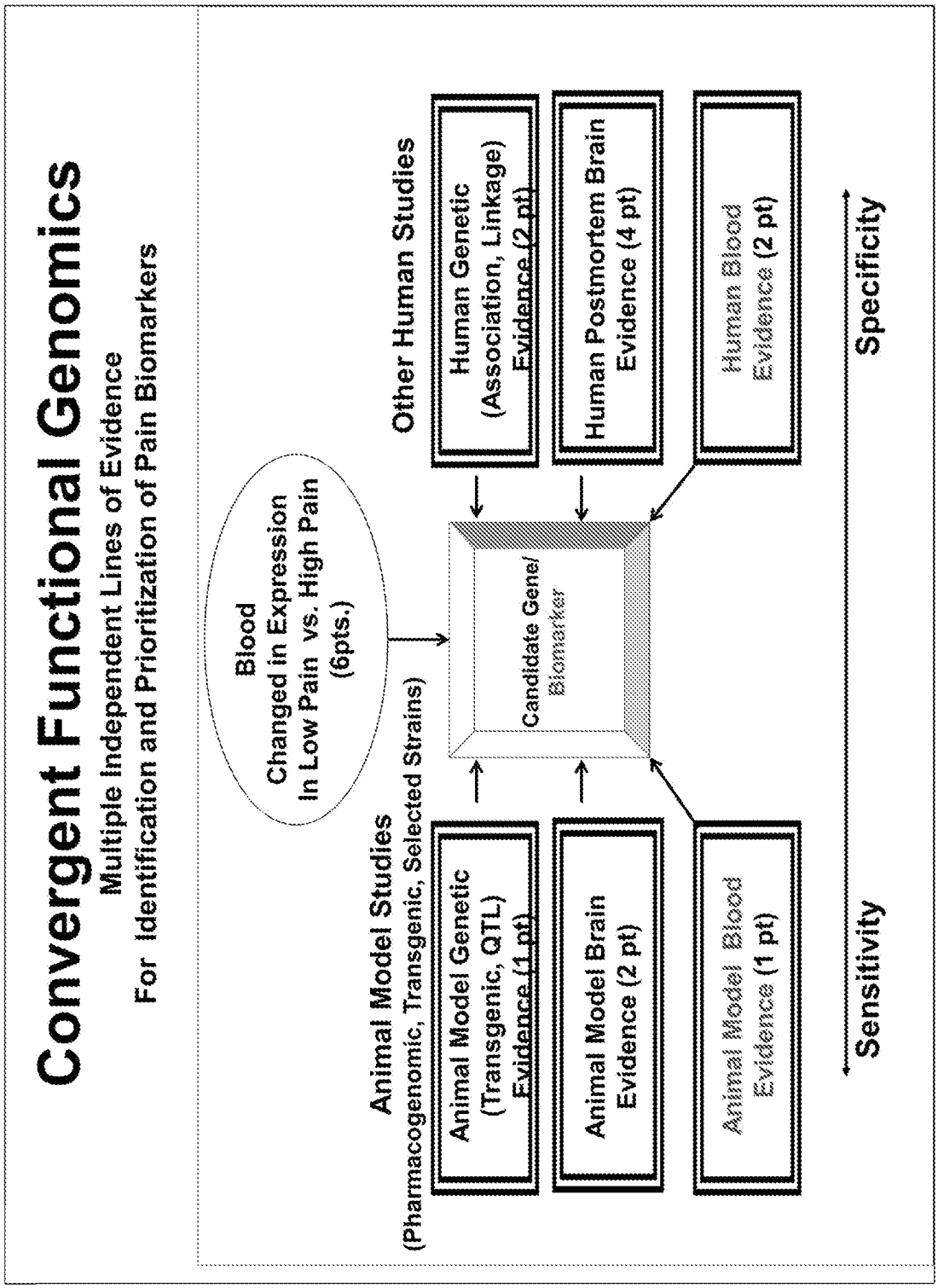

Convergent Functional Genomics
Multiple Independent Lines of Evidence
For Identification and Prioritization of Pain Biomarkers
Blood
Changed in Expression
In Low Pain vs. High Pain
(6pts.)
Animal Model Studies
(Pharmacogenomic, Transgenic, Selected Strains)
Animal Model Genetic
(Transgenic, QTL)
Evidence (1 pt)
Animal Model Brain
Evidence (2 pt)
Animal Model Blood
Evidence (1 pt)
Candidate Gene/
Biomarker
Other Human Studies
Human Genetic
(Association, Linkage)
Evidence (2 pt)
Human Postmortem Brain
Evidence (4 pt)
Human Blood
Evidence (2 pt)
Sensitivity
Specificity

FIG. 1E

FIG. 1F

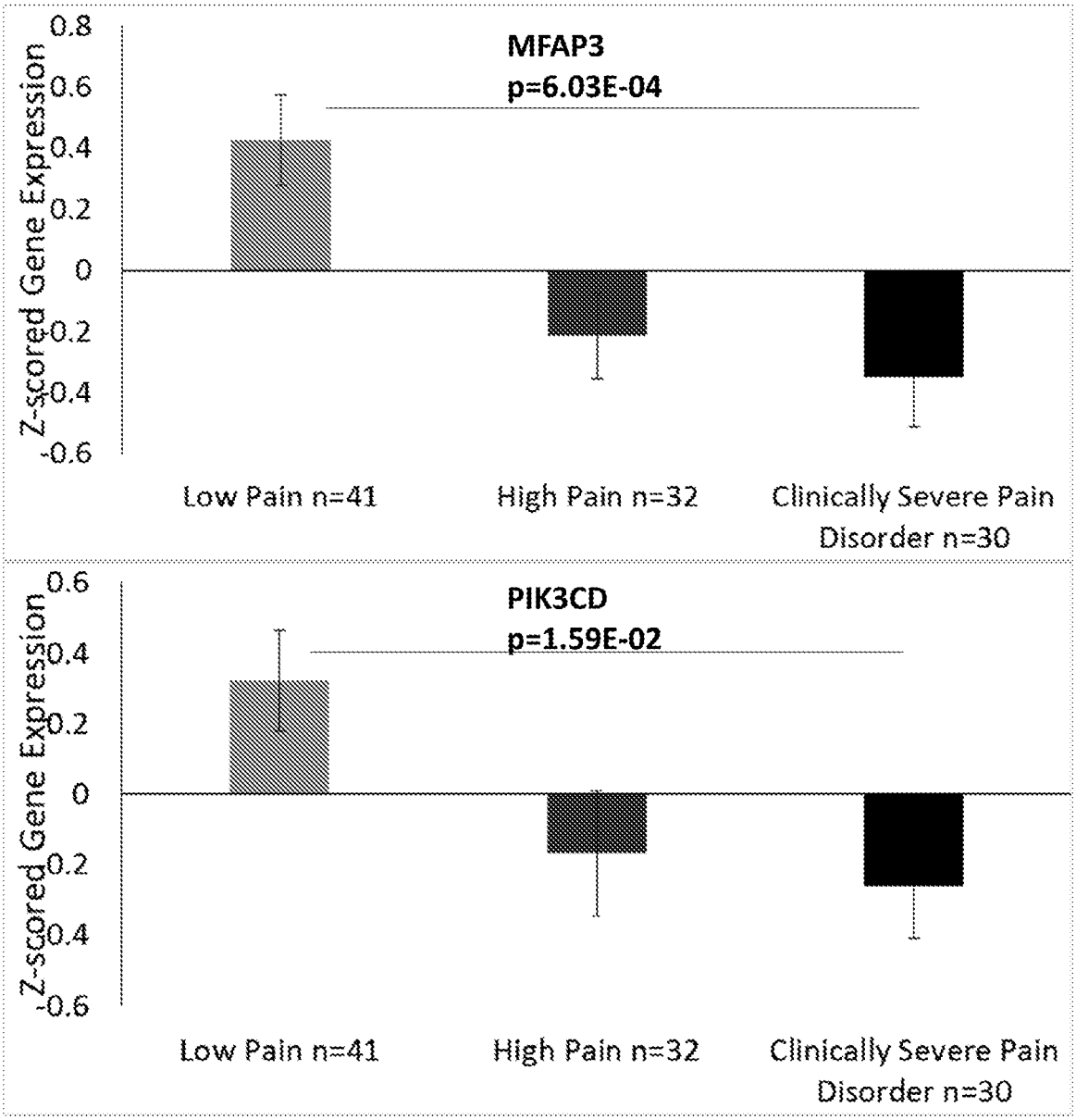

MFAP3
p=6.03E-04
Z-scored Gene Expression
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Low Pain n=41
High Pain n=32
Clinically Severe Pain Disorder n=30
PIK3CD
p=1.59E-02
Z-scored Gene Expression
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Low Pain n=41
High Pain n=32
Clinically Severe Pain Disorder n=30

FIG. 1G

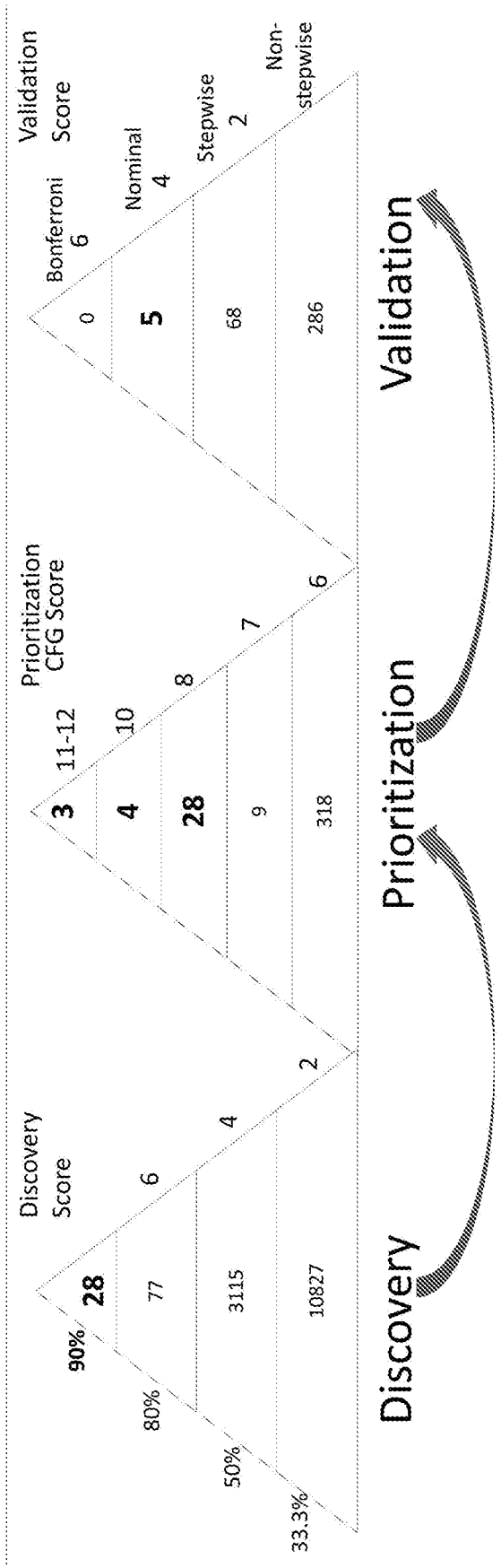
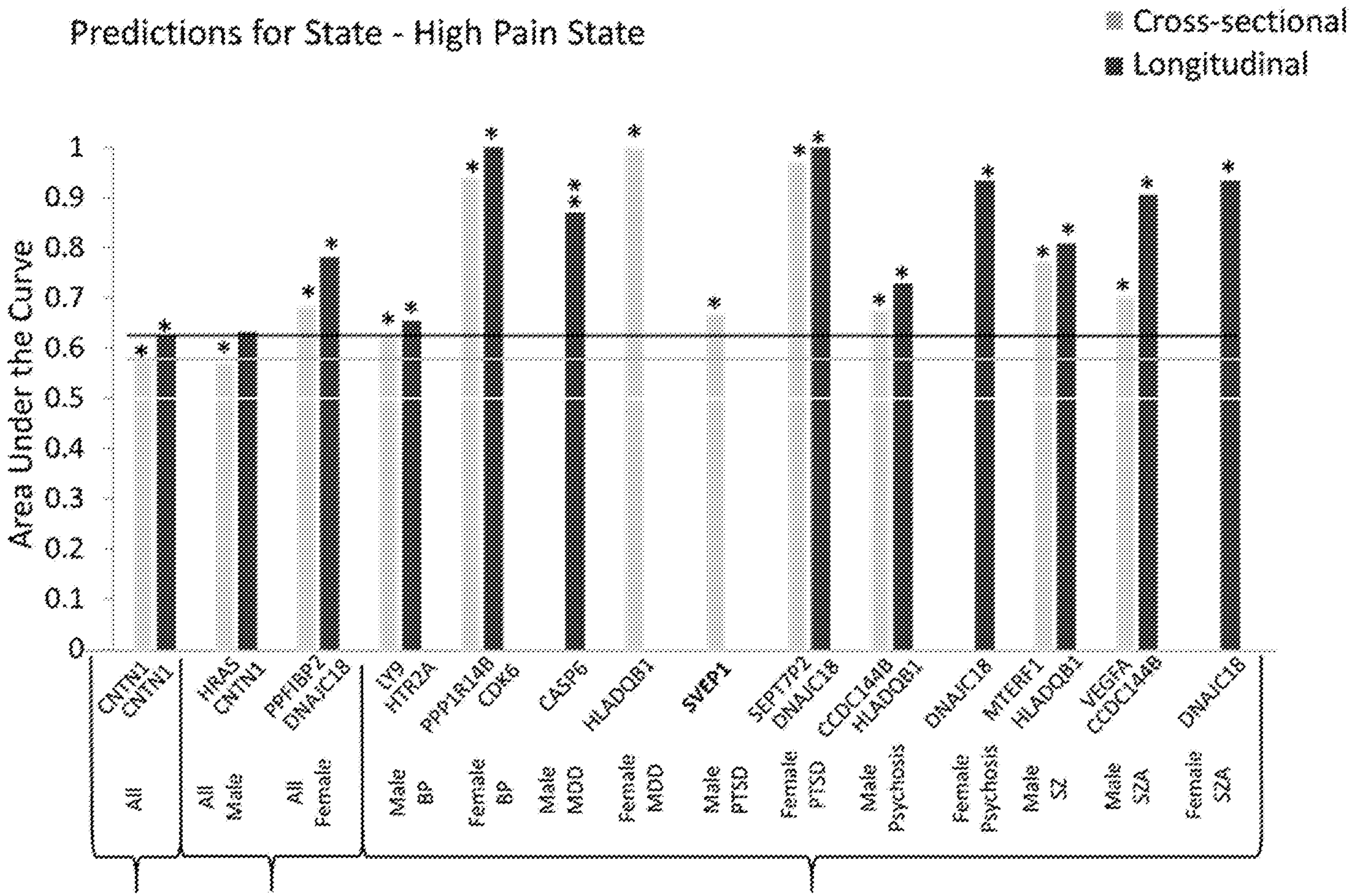
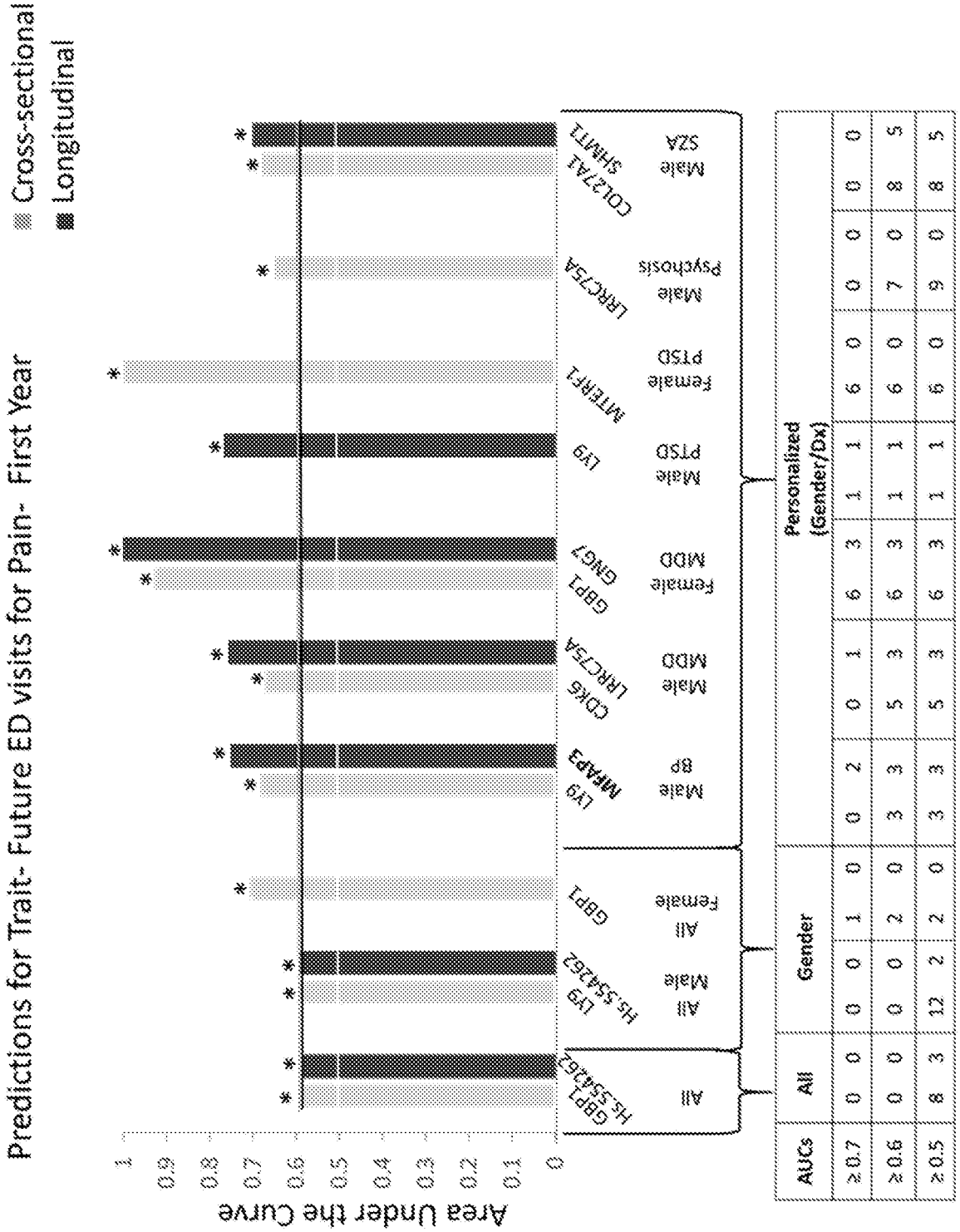

Discovery
Score
90%
80%
50%
33.3%
28
6
4
2
77
3115
10827
Prioritization
CFG Score
3
11-12
10
8
7
6
4
28
9
318
Validation
Score
Bonferroni
6
Nominal
4
Stepwise
2
Non-
stepwise
0
5
68
286
Discovery
Prioritization
Validation

FIG. 2A



AUCs	All	Gender		Personalized (Gender/Dx)											
≥0.7	0 0	0 0	0 2	0 0	7 6	0 9	17 0	0 2	4 2	0 1	0 1	4 6	1 3	NA 1	
≥0.6	0 1	0 3	5 3	2 2	7 6	0 17	17 0	1 2	4 2	4 3	0 1	10 6	1 3	NA 1	
≥0.5	4 3	3 4	5 3	2 2	7 6	0 17	17 0	1 2	4 2	4 3	0 1	10 6	1 3	NA 1	

FIG. 2B



AUCs	All	Gender			Personalized (Gender/Dx)									
≥0.7	0	0	0	0	0	2	0	1	6	3	1	1	6	0	0	0	0	0
≥0.6	0	0	0	0	2	0	3	3	5	3	1	1	6	0	7	0	8	5
≥0.5	8	3	12	2	2	0	3	3	5	3	6	3	1	1	6	0	9	0	8	5

FIG. 2C

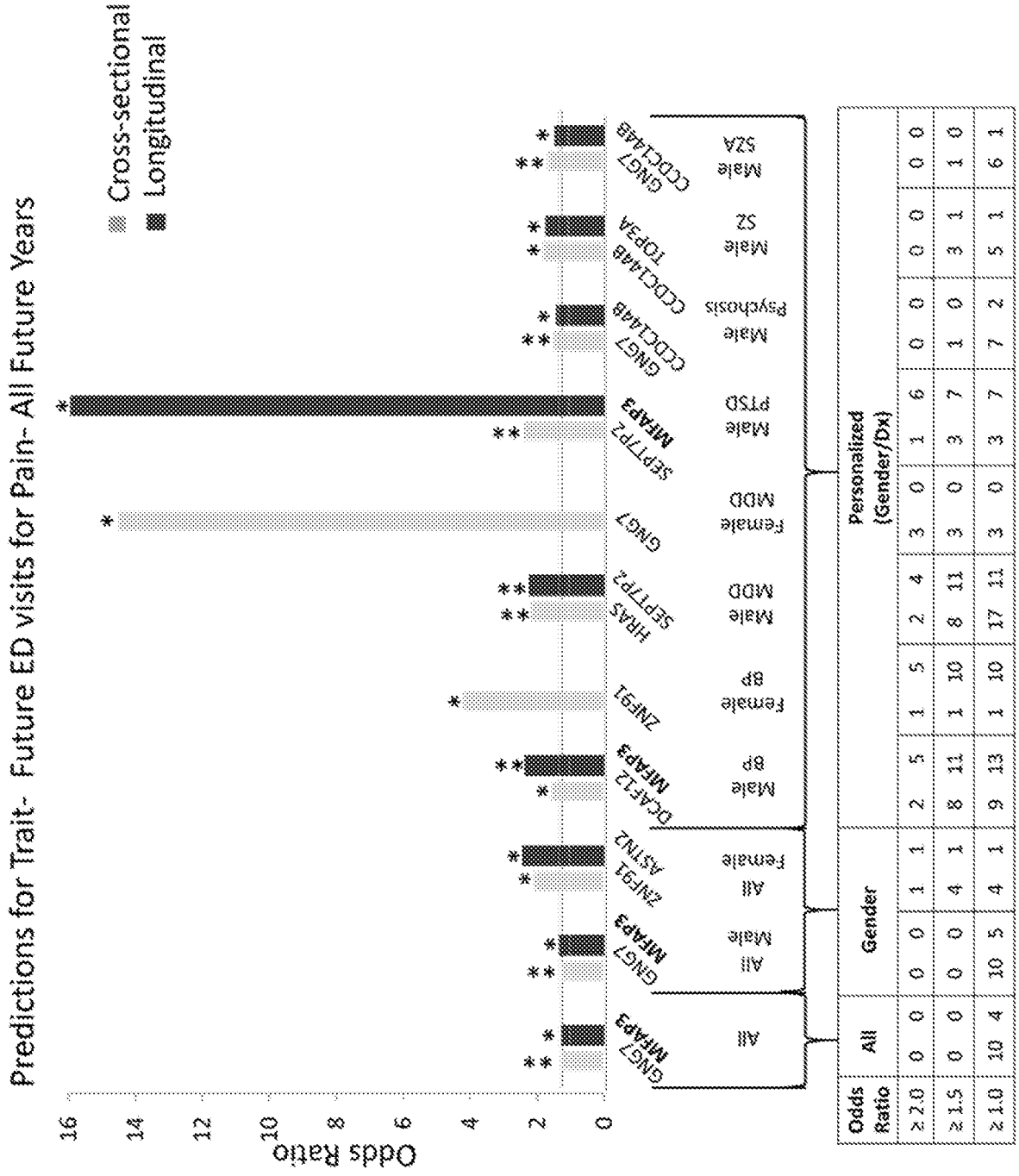
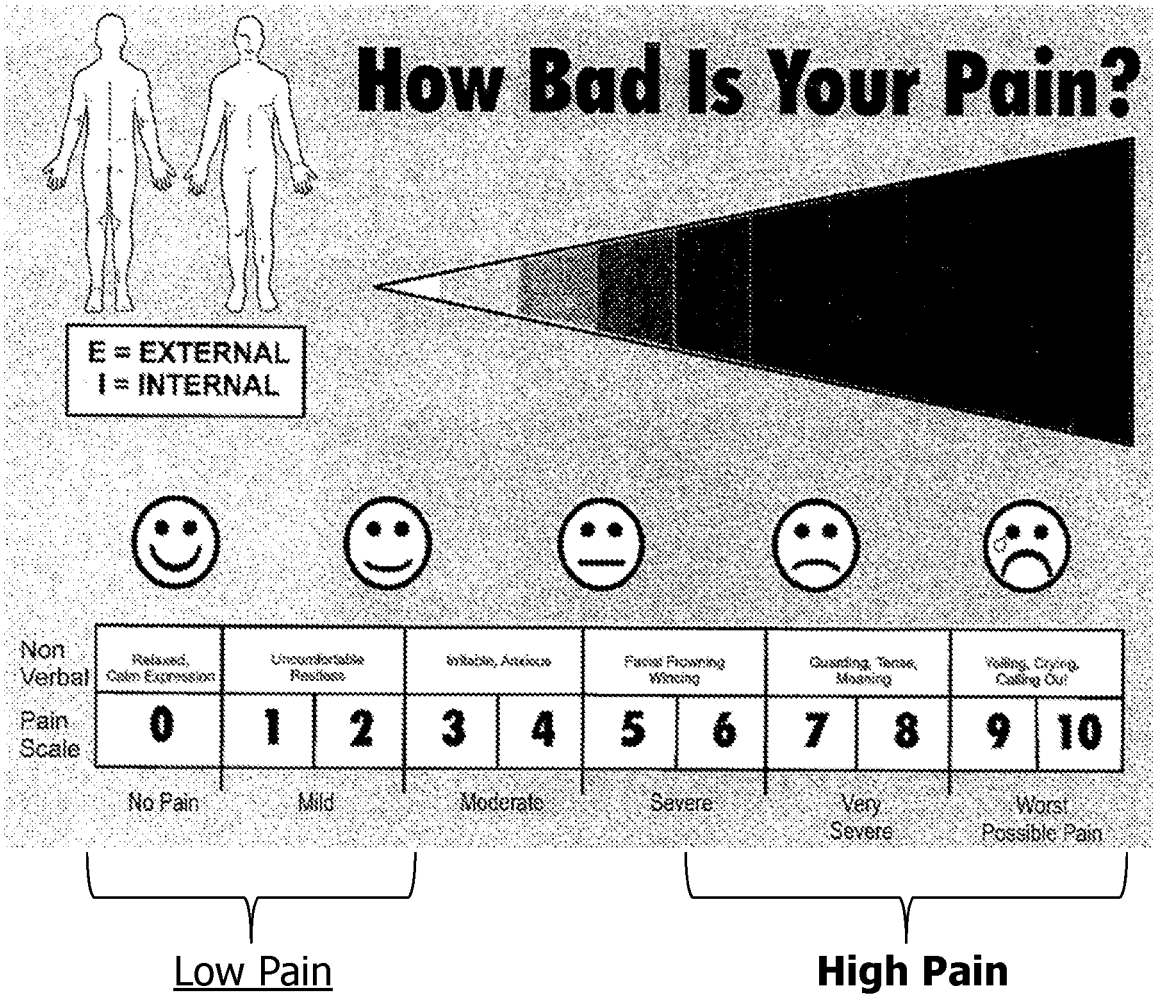

Predictions for Trait- Future ED visits for Pain- All Future Years
Cross-sectional
Longitudinal
Odds Ratio
0
2
4
6
8
10
12
14
16
GNG7 MIFAP3
All
All
GNG7 MIFAP3
All Male
ZNF91 AS7NZ
All Female
DCAF12 MIFAP3
Male BP
ZNF91
Female BP
HRAS SEPTTP2
Male MDD
GRG7
Female MDD
SEPTTP2 MIFAP3
Male PTSD
GNG7 CCDC144B
Male Psychosis
TOP3A CCDC144B
Male S2
GNG7 CCDC144B
Male S2A
Odds Ratio
All
Gender
Personalized (Gender/Dx)
≥ 2.0 0 0 0 0 1 1 2 5 1 5 2 4 3 0 1 6 0 0 0 0 0 0
≥ 1.5 0 0 0 0 4 1 8 11 1 10 8 11 3 0 3 7 1 0 3 1 1 0
≥ 1.0 10 4 10 5 4 1 9 13 1 10 17 11 3 0 3 7 7 2 5 1 6 1

FIG. 3

Discovery Cohort:
19 male and 9 female psychiatric participants who have at least one switch between a Low Pain state visit and a **High Pain** state visit.



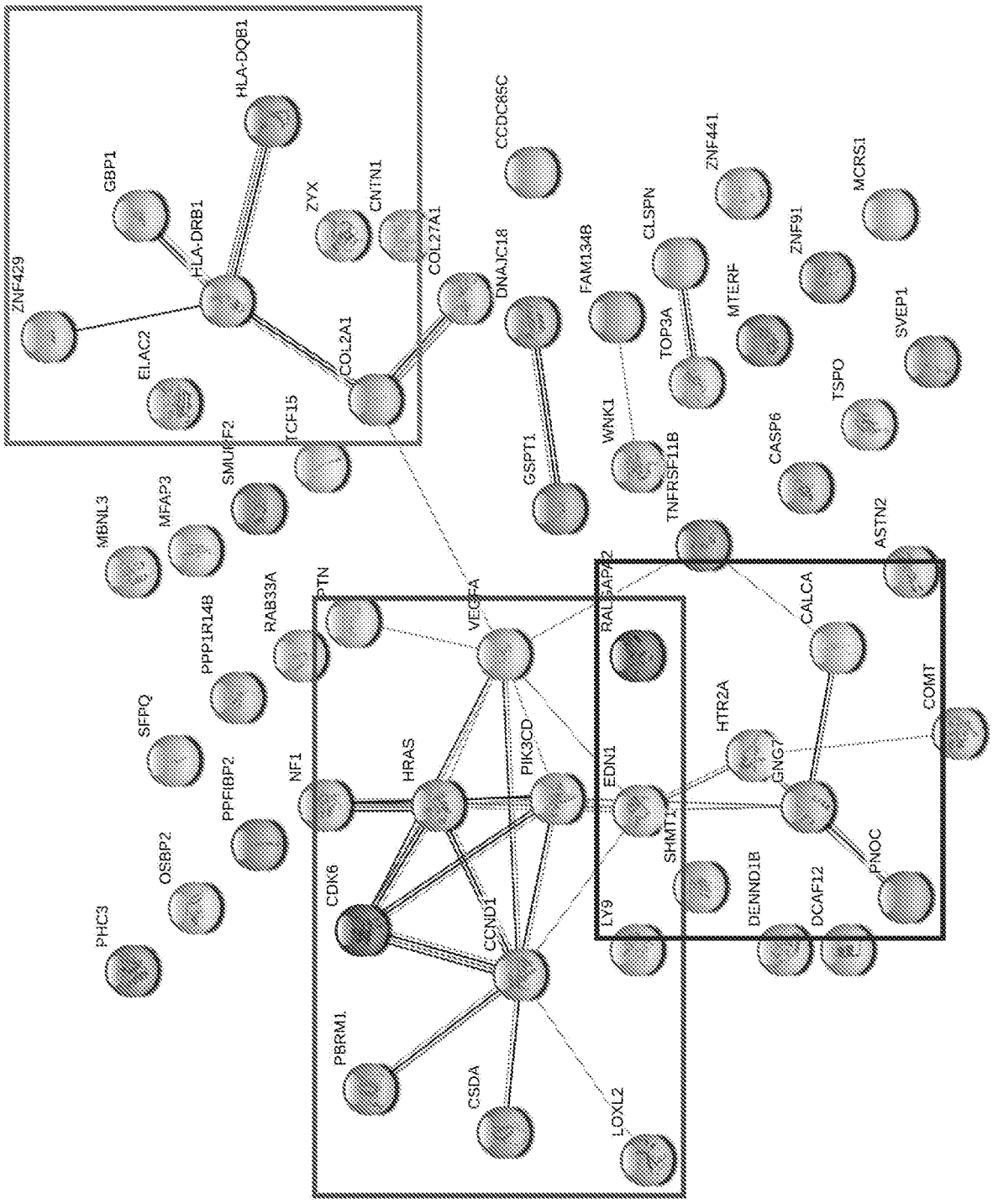

HLA-DQB1
GBP1
HLA-DRB1
ZYX
CNTN1
ZNF429
COL27A1
CCDC85C
DNAJC18
FAM134B
CLSPN
ZNF441
ELAC2
COL2A1
MTERF
ZNF91
MCRS1
TOP3A
TCF15
SMURF2
GSPT1
WNK1
TSPO
SVEP1
CASP6
MBNL3
MFAP3
TNFRSF11B
ASTN2
PTN
VEGFA
RALBP1
CALCA
RAB33A
PPP1R14B
SFPQ
NF1
HRAS
PIK3CD
EDN1
HTR2A
COMT
PPFIBP2
GNG7
OSBP2
CDK6
SHMT1
PHC3
CCND1
LY9
DENND1B
DCAF12
PNOC
PBRM1
CSDA
LOXL2

FIG. 4