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(54) Title: METHODS FOR IDENTIFYING COMPOUNDS SUITABLE FOR TREATMENT OF CENTRAL NERVOUS SYSTEM TRAUMA AND USES OF THOSE COMPOUNDS

(57) Abstract: Provided are uses of and methods of treatment involving a sulfonamide for treatment of central nervous system trauma, including but not limited to a spinal cord injury and/or a traumatic brain injury. The central nervous system trauma may be a primary injury or may be a secondary injury. Also provided are methods of identifying a compound suitable for treatment of central nervous system trauma.

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Methods for Identifying Compounds Suitable for Treatment of Central Nervous System Trauma and Uses of Those Compounds

TECHNICAL FIELD

The present invention relates to the field of neurotraumatic injury, including spinal cord injury (SCI) and traumatic brain injury (TBI). More specifically, the invention relates to methods for selecting chemical compounds for use in treating or improving neurological outcome after central nervous system trauma.

BACKGROUND

Spinal cord injury (SCI) is a devastating neurological condition for which there are currently no effective treatment options. Over 250,000 individuals in the world sustain a traumatic spinal cord injury every year (Devivo MJ, (2012 - Epub 2012 Jan 24). 'Epidemiology of traumatic spinal cord injury: trends and future implications', *Spinal Cord* 50(5):365-72. doi: 10.1038/sc.2011.178.), primarily from motor vehicle accidents and falls (DeVivo MJ (1997) 'Causes and costs of spinal cord injury in the United States'. *Spinal Cord* 35:809). Presently there are over 2.5 million people living with SCI worldwide (Ackery A, *et al.*, (2004) 'A global perspective on spinal cord injury epidemiology' *J Neurotrauma* 21:1355–1370). The lifetime healthcare expenditures for individuals with SCI are among the most expensive of any medical condition, and are estimated to be near \$1 million for a paraplegic person and \$2.9 million for a tetraplegic person (Krueger H, *et al.*, (2013), 'The economic burden of traumatic spinal cord injury in Canada', *Chronic Dis Inj Can.*33(3):113-22). Canadian data show substantial costs during the first year of care, estimating more than \$120,000 per person with complete SCI and more than \$40,000 with incomplete SCI, with higher costs in subsequent years (Dryden DM, *et al.*, (2005) 'Direct health care costs after traumatic spinal cord injury' *J Trauma* 59:443–449).

SCI results in the impairment of motor, sensory, and autonomic systems, causing profound dysregulation of almost every bodily function. The failure of large-scale clinical trials of drug therapies in acute SCI (Bracken MB, *et al.*, (1990) 'A Randomized, Controlled Trial of Methylprednisolone or Naloxone in the Treatment of Acute Spinal-

Cord Injury' *N Engl J Med* 322:1405–1411; Geisler FH, *et al.*, (2001) 'The Sygen multicenter acute spinal cord injury study' *Spine* (Phila Pa 1976) 26:S87-98), and the lack of success in translating preclinical therapies to humans (Ramer LM, *et al.*, (2014) 'Restoring function after spinal cord injury: Towards clinical translation of experimental strategies' *Lancet Neurol* 13:1241–1256), leaves clinicians without effective treatment options for SCI. There are currently only two methods to improve neurological outcome after SCI: early decompression (Fehlings MG, *et al.*, (2012) 'Early versus delayed decompression for traumatic cervical spinal cord injury: Results of the surgical timing in acute spinal cord injury study' (STASCIS) Di Giovanni S, ed. *PLoS One* 7:e32037) and hemodynamic optimization (Vale FL, *et al.*, (1997) 'Combined medical and surgical treatment after acute spinal cord injury: results of a prospective pilot study to assess the merits of aggressive medical resuscitation and blood pressure management' *J Neurosurg* 87:239–246). However, both methods are limited as the observed improvement, even at its best, does not often provide significant improvement in functional ability for the individual. The fact that no effective treatment for SCI has emerged reflects the complexity of the pathophysiologic mechanisms activated by central nervous system (CNS) injury. The additive effects of the immune response (Demjen D, *et al.*, (2004) 'Neutralization of CD95 ligand promotes regeneration and functional recovery after spinal cord injury' *Nat Med* 10:389–395; Kigerl KA, *et al.*, (2009) 'Identification of Two Distinct Macrophage Subsets with Divergent Effects Causing either Neurotoxicity or Regeneration in the Injured Mouse Spinal Cord' *J Neurosci* 29:13435–13444), a cascade of apoptotic and necrotic processes (Crowe MJ, *et al.*, (1997) 'Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys' *Nat Med* 3:73–76; Springer JE, *et al.*, (1999) 'Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury' *Nat Med* 5:943–946), neuronal growth suppression (Schnell L, *et al.*, (1990) 'Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors' *Nature* 343:269–272; GrandPre T, *et al.*, (2000) 'Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein' *Nature* 403:439–444), and the formation of an inhibitory glial scar (Bradbury EJ, *et al.*, (2002) 'Chondroitinase ABC promotes functional recovery after spinal cord injury' *Nature* 416:636–640) pose a challenge to the

development of new therapeutic strategies. Despite the damage caused by both the primary and secondary injuries, it is well known that few SCIs result in the complete destruction of all ascending and descending fibers (Kakulas BA (1999) 'A review of the neuropathology of human spinal cord injury with emphasis on special features' *J Spinal Cord Med* 22:119). Therapies targeted at preventing secondary damage and preserving maximal amounts of neural tissue are therefore crucial and while a number of compounds have been investigated for their role in spinal cord injury, few have made it to clinical trial, and none have made it into clinical practice.

SUMMARY

In illustrative embodiments of the present invention, there is provided, use of a sulfonamide for treatment of central nervous system trauma.

In illustrative embodiments of the present invention, there is provided a use described herein, wherein the sulfonamide is selected from the group consisting of: Acetazolamide, Acetohexamide, Amprenavir, Apricoxib, Asunaprevir, Azabon, Beclabuvir, Bosentan, Brinzolamide, Bumetanide, Carbutamide, Celecoxib, Chlorpropamide, Chlorthalidone, Clopamide, Darunavir, Dasabuvir, Delavirdine, Dofetilide, Dorzolamide, Dronedarone, Ethoxzolamide, Fosamprenavir, Furosemide, Glibenclamide, Glibornuride, Gliclazide, Glycropyramide, Glimepiride, Glipizide, Gliquidone, Glisoxepide, Grazoprevir, Hydrochlorothiazide, Ibutilide, Indapamide, Mafenide, Mefruside, Methazolamide, Metolazone, Parecoxib, Paritaprevir, Probenecid, Simeprevir, Sotalol, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfametopyrazine, Sulfametoxydiazine, Sulfamoxole, Sulfanitran, Sulfaphenazole, Sulfasalazine, Sulfisomidine, Sultiame, Sumatriptan, Tamsulosin, Terephtyl, Tipranavir, Tolazamide, Tolbutamide, Udenafil, Xipamide, and Zonisamide.

In illustrative embodiments of the present invention, there is provided a use described herein, wherein the sulfonamide is sulfaphenazole.

In illustrative embodiments of the present invention, there is provided a use described herein, wherein the central nervous system trauma is a spinal cord injury.

In illustrative embodiments of the present invention, there is provided a use described herein, wherein the central nervous system trauma is a traumatic brain injury.

In illustrative embodiments of the present invention, there is provided a use described herein, wherein the central nervous system trauma is a primary injury or a secondary injury.

In illustrative embodiments of the present invention, there is provided a method of identifying a compound suitable for treatment of central nervous system trauma, the method comprising: (a) providing a subject having central nervous system trauma wherein a severity-dependent gene subnetwork is upregulated in the subject; (b) administering a test compound to the subject; and (c) determining whether the upregulation of the severity-dependent gene subnetwork is reversed in the presence of the of the test compound, wherein the reversal of the upregulation is in indication that the test compound is suitable for treatment of central nervous system trauma.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is selected from the group consisting of: M1, M2, M3, M6, M7, M8, and M11.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is M3 or M7.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is M3.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the central nervous system trauma is spinal cord injury.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the severity-dependent gene subnetwork is a brain cortex gene subnetwork and is M9.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the central nervous system trauma is a traumatic brain injury.

In illustrative embodiments of the present invention, there is provided a method described herein wherein the central nervous system trauma is a primary injury or a secondary injury.

In illustrative embodiments of the present invention, there is provided use of a compound identified using a method described herein for treatment of central nervous system trauma.

In illustrative embodiments of the present invention, there is provided a method of medical treatment comprising administering a compound identified using a method described herein to a patient having central nervous system trauma.

In illustrative embodiments of the present invention, there is provided a method of medical treatment comprising administering a sulfonamide to a patient having central nervous system trauma.

In illustrative embodiments of the present invention, there is provided a method of medical treatment described herein wherein the sulfonamide is selected from the group consisting of: Acetazolamide, Acetohexamide, Amprenavir, Apricoxib, Asunaprevir, Azabon, Beclabuvir, Bosentan, Brinzolamide, Bumetanide, Carbutamide, Celecoxib, Chlorpropamide, Chlorthalidone, Clopamide, Darunavir, Dasabuvir, Delavirdine, Dofetilide, Dorzolamide, Dronedarone, Ethoxzolamide, Fosamprenavir, Furosemide, Glibenclamide, Glibornuride, Gliclazide, Glyclopamide, Glimepiride, Glipizide, Gliquidone, Glisoxepide, Grazoprevir, Hydrochlorothiazide, Ibutilide, Indapamide, Mafenide, Mefruside, Methazolamide, Metolazone, Parecoxib, Paritaprevir, Probenecid, Simeprevir, Sotalol, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfametopyrazine, Sulfametoxydiazine, Sulfamoxole, Sulfanitran, Sulfaphenazole, Sulfasalazine, Sulfisomidine, Sultiame, Sumatriptan, Tamsulosin, Terephtyl, Tipranavir, Tolazamide, Tolbutamide, Udenafil, Xipamide, and Zonisamide.

In illustrative embodiments of the present invention, there is provided a method of medical treatment described herein wherein the sulfonamide is sulfaphenazole.

In illustrative embodiments of the present invention, there is provided a method of medical treatment described herein wherein the central nervous system trauma is a spinal cord injury.

In illustrative embodiments of the present invention, there is provided a method of medical treatment described herein wherein the central nervous system trauma is a traumatic brain injury.

In illustrative embodiments of the present invention, there is provided a method of medical treatment described herein wherein the central nervous system trauma is a primary injury or a secondary injury.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Literature curation and validation of genes implicated in the physiological response to spinal cord injury (SCI) by small-scale experiments. (A) Number of small-scale studies implicating each gene in SCI pathophysiology in the literature-curated (LC) gene set. (B) Experimental techniques used to associate LC genes with response to SCI in the LC gene set. (C) Enrichment for shared Gene Ontology terms among LC genes. (D) Number of protein-protein interactions (PPIs) between LC genes observed in the high-confidence human interactome (dotted line) and 1,000 randomized interactome networks (density). (E) Size of the largest connected component (LCC) between LC genes in the high-confidence human interactome (dotted line) and 1,000 randomized interactome networks (density). (F) LC genes are prioritized by a disease gene prediction algorithm (Maslov S, *et al.*, (2002) 'Specificity and Stability in Topology of Protein Networks' *Science* (80-) 296:910–913).

Figure 2: Validation of the complete set of genes implicated in the physiological response to SCI. (A) Experimental models of SCI employed to associate LC genes with response to SCI in the LC gene set. (B) Species in which LC genes were associated with response to SCI in the LC gene set. (C) Time points at which LC genes were associated with response to SCI in the LC gene set. (D–E) Number of binary (D) or co-complex (E) protein-protein interactions (PPIs) between LC genes observed in a second high-quality human interactome (Li T, *et al.*, (2017) 'A scored human protein-protein interaction network to catalyze genomic interpretation' *Nat Methods* 14:61–64).

(dotted line) and 1,000 randomized interactome networks (density). (F–G) Size of the largest connected component (LCC) between LC genes in binary (F) or co-complex (G) high-quality human interactomes (dotted line) and 1,000 randomized interactome networks (density). (H) Number of intra-complex co-memberships between LC genes (dotted line) and 1,000 randomized gene sets (density) observed in a global map of human protein complexes (Drew K, *et al.*, (2016) 'A synthesis of over 9,000 mass spectrometry experiments reveals the core set of human protein complexes' *bioRxiv*:92361). (I) LC genes are prioritized by a disease gene prediction algorithm (Ghiassian SD, *et al.*, (2015) 'A Disease Module Detection (DIAMOnD) Algorithm Derived from a Systematic Analysis of Connectivity Patterns of Disease Proteins in the Human Interactome Rzhetsky A', ed. *PLOS Comput Biol* 11:e1004120) in an interactome including orthologous interactions detected in model organisms (Li T, *et al.*, (2017) (*ibid*)).

Figure 3: Gene coexpression modules in the human spinal cord and their differential expression in SCI. (A) Reproducibility of human spinal cord modules in a microarray dataset and conservation in mouse and rat. (B) Enrichment of M3 and M7 for LC SCI genes. (C) Robustness of M3 and M7 enrichment for LC SCI genes. (D) Eigengene network for human spinal cord modules. (E) Differential expression of spinal cord modules following SCI in five datasets, and consensus. (F) Evidence for differential expression of six consensus modules and one majority module (M8). (G) Time-dependent expression of spinal cord modules at acute, subacute, and chronic time points following SCI.

Figure 4: Biological characterization of spinal cord modules. (A–B) Enrichment maps for modules M3 and M7. (C) Meta-analysis of cell type-specific marker gene enrichment in human spinal cord modules at the transcriptomic and proteomic levels.

Figure 5: Relationship of spinal cord modules to injury severity and motor and sensory functional recovery. (A) Enrichment of spinal cord modules for genes correlated or anticorrelated to injury severity in a mouse model. (B) Consensus network signature of SCI pathophysiology, validation in independent transcriptomic and proteomic datasets, and reversal in functional recovery. (C) Gene expression

correlation to M3 eigengene predicts association to SCI severity. (D) Reproducibility and evolutionary conservation of spinal cord modules and their preservation at the proteomic level. (E–F) Relationship between M3 eigengene and injury severity at 7 days post-injury in a mouse model (E), and in a novel RNA-seq (F) and proteomics (G) datasets. (H) Downregulation of the M3 eigengene following treatment with NT-3, a neurotrophic agent that promotes functional recovery in acute SCI. (I) Six genes classify moderate and severe injuries in transcriptomic data with 90% or greater accuracy. (J–K) Gene expression and protein abundance of annexin A1 in sham, moderate, and severe SCI.

Figure 6: Consensus network signature of SCI pathophysiology, validation in independent transcriptomic and proteomic datasets, and reversal in functional recovery and reduced axonal dieback (Squair *et al.*, (2018), 'Integrated systems analysis reveals conserved gene networks underlying response to spinal cord injury', *Elife* 2;7. pii: e39188. doi: 10.7554/eLife.39188).

Figure 7: A) Computational predictions of drugs from the CMap database that would significantly reduce the expression of M3, associated with spinal cord injury, with statistical significance derived from a Wilcoxon rank-sum test. B) Computational predictions of drugs from the CMap database that would significantly reduce the expression of M9, associated with traumatic brain injury, with statistical significance derived from a Wilcoxon rank-sum test.

Figure 8: A) Consensus network signature of SCI pathophysiology, validation in independent transcriptomic and proteomic datasets, and reversal after treatment with sulfaphenazole. B) Consensus network signature of traumatic brain injury (TBI) pathophysiology.

Figure 9: Demonstration that sulfaphenazole improves cardiovascular function. Top panels represent individual animal responses in rats subjected to sham injury, T3 SCI or T3 SCI plus daily sulfaphenazole (SP). Additionally shown are the group mean data for systolic blood pressure (SBP), dP/dT_{max} , and end-systolic elastance (Ees). * $P < 0.05$; Error bars represent standard error of the mean

Figure 10: GSEA plot of M3 in the RNA-seq data. Enrichment of M3 genes among genes downregulated in the spinal cord parenchyma of rats subjected to

experimental spinal cord injury treated with sulfaphenazole as compared to untreated injured rats, as discerned by RNA sequencing.

Figure 11: GSEA plot of M3 in proteomics data. Enrichment of M3 genes among proteins downregulated in the spinal cord parenchyma of rats subjected to experimental spinal cord injury treated with sulfaphenazole as compared to untreated injured rats, as discerned by untargeted preteomics.

DETAILED DESCRIPTION

As used herein, “a sulfonamide” is a pharmaceutical of the general formula of $R^1-SO_2-N(R^2)(R^3)$. The functional group “ SO_2-N ” is the basis of several groups of pharmaceuticals, referred to herein as sulfonamides. Some sulfonamides are antibacterial. Some sulfonamides are not antibacterial. Some non-limiting examples of sulfonamides include: Acetazolamide, Acetohexamide, Amprenavir, Apricoxib, Asunaprevir, Azabon, Beclabuvir, Bosentan, Brinzolamide, Bumetanide, Carbutamide, Celecoxib, Chlorpropamide, Chlorthalidone, Clopamide, Darunavir, Dasabuvir, Delavirdine, Dofetilide, Dorzolamide, Dronedarone, Ethoxzolamide, Fosamprenavir, Furosemide, Glibenclamide, Glibornuride, Gliclazide, Glyclopamide, Glimepiride, Glipizide, Gliquidone, Glisoxepide, Grazoprevir, Hydrochlorothiazide, Ibutilide, Indapamide, Mafenide, Mefruside, Methazolamide, Metolazone, Parecoxib, Paritaprevir, Probenecid, Simeprevir, Sotalol, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfametopyrazine, Sulfametoxydiazine, Sulfamoxole, Sulfanitran, Sulfaphenazole, Sulfasalazine, Sulfisomidine, Sultiame, Sumatriptan, Tamsulosin, Terephtyl, Tipranavir, Tolazamide, Tolbutamide, Udenafil, Xipamide, and Zonisamide.

As used herein “central nervous system trauma” or “CNS trauma” refers to a diverse group of disorders that include spinal cord injury (SCI) and traumatic brain injury (TBI). Central nervous system trauma is characterized by a mechanical impact, often in a car accident or other situations resulting in blows to the head or spine, that results in a mechanical response at the cellular and tissue level that leads to a pathophysiological response. In some cases the mechanical impact may be from a tumor or other

biological entity physically invading the central nervous system. In such traumas, there is often an acute phase followed by a chronic phase.

As used herein “spinal cord injury” or “SCI” refers to damage to the spinal cord that causes temporary or permanent changes in its function. Symptoms may include loss of muscle function, sensation, or autonomic function in the parts of the body served by the spinal cord below the level of the injury. Injury can occur at any level of the spinal cord and can be complete injury, with a total loss of sensation and muscle function, or incomplete, meaning some nervous signals are able to travel past the injured area of the cord. Depending on the location and severity of damage, the symptoms vary, from numbness to paralysis to incontinence. Complications can include muscle atrophy, pressure sores, infections, and breathing problems.

As used herein “traumatic brain injury” or “TBI” refers to a nondegenerative, noncongenital insult to the brain from an external mechanical force, possibly leading to permanent or temporary impairment of cognitive, physical, and psychosocial functions, with an associated diminished or altered state of consciousness.

As used herein “a primary injury” refers to the damage occurring from central nervous system trauma wherein cell death occurs immediately from the original injury.

As used herein “a secondary injury” refers to damage occurring from a central nervous system trauma wherein biochemical cascades that are initiated by the primary injury cause further tissue damage. These secondary injury pathways include the ischemic cascade, inflammation, swelling, cell suicide, and neurotransmitter imbalances. They can take place for minutes or weeks following the injury.

As used herein “a subject” refers to an animal, including but not limited to a human, a mouse, a rat, a pig, a primate, and other mammals, that has a spinal cord and a central nervous system.

As used herein “a severity-dependent gene subnetwork” refers to a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins. The particular subnetwork will only be active if the severity of the central nervous system trauma is of a particular severity, while another subnetwork will be active if the central nervous system trauma is of a different severity. The regulators in a subnetwork may be DNA, RNA, protein and

complexes of these. The interaction between components of the subnetwork may be direct or indirect.

As used herein “a field standard contusion spinal cord injury (SCI)” refers to damaging the spinal cord using blunt force in the form of a servo-controlled impactor, forceps, or using a weight dropped from a given height or similar mechanism known to and understood by a person of skill in the art.

The invention described herein relates to the selection of pharmaceuticals for use in the treatment of spinal cord injury (SCI) as well as to the selection of new therapeutic indications for existing pharmaceuticals. Embodiments of the invention relate to methods for treating a subject with neurotraumatic injury and/or CNS trauma, including but not limited to SCI and TBI, by administering a pharmaceutical identified herein. It is to be understood that the present invention may be embodied in various forms. Therefore, the specific details disclosed herein are not to be interpreted as limiting, but rather as a representative basis for teaching one of skill in the art how to employ the present invention in any detailed system, structure, or manner.

The invention relates, at least in part, to selection of known therapeutic compounds and methods of use for the selected compounds in treating neurotraumatic injury and/or CNS trauma, including but not limited to SCI and TBI, in a subject.

In one embodiment of the invention, there is provided a method of treatment for a CNS trauma, the method including: identifying a compound for use in treating a CNS trauma, and administering said compound to a subject in need thereof. The method of identifying a compound for use in treating a CNS trauma may include the following: identifying a subnetwork of genes associated with a CNS trauma, identifying genes within the subnetwork wherein gene expression is upregulated following a CNS trauma and down-regulated during functional recovery, and identifying a compound that reverses the expression patterns of the identified genes. The reversal of gene expression patterns may be for the treatment of SCI. The reversal of gene expression patterns may be for the treatment of TBI. The treatment of a CNS trauma may include reducing the extent of neurotraumatic injury. The treatment of a CNS trauma may include promoting repair by, for example, improving blood flow, improving tissue perfusion etc. The CNS trauma may be a primary injury and/or a secondary injury. In

some embodiments, the compound administered to the subject may be a sulfonamide. In some embodiments, the compound may be sulfaphenazole.

In one embodiment of the invention, there is provided a use of a compound for the treatment of a CNS trauma. The CNS trauma may include a primary injury and/or a secondary injury. The compound may be for use in treating SCI. The compound may be for use in treating TBI. The treatment of CNS trauma may include reducing the extent of neurotraumatic injury. The treatment of CNS trauma may include promoting repair by, for example, improving blood flow, improving tissue perfusion etc. In one embodiment, the compound for use in treating a CNS trauma may be a sulfonamide. The compound may be sulfaphenazole.

In one embodiment of the invention, there is provided a use of a compound for modulating expression activity of a gene subnetwork. The gene subnetwork may be the M3 gene subnetwork. Modulation of expression activity may include an up/down regulation of i) the module eigengene (first principal component of module gene expression), or ii) that M3 genes would be over-represented or enriched when genes are ranked by statistical coefficient used to perform differential expression analysis (GSEA), or iii) an increase in expression of one or more genes in the M3 subnetwork. Modulation of expression activity may include a decrease in gene expression of one or more genes in the M3 subnetwork. Modulation of expression activity may include an increase in expression activity of one or more genes in the M3 subnetwork and a decrease in expression activity of one or more genes in the M3 subnetwork.

In one embodiment of the invention, there is provided a use of a compound for the manufacture of a medicament for modulating expression activity of the M3 gene subnetwork. Modulation of expression activity may include an increase in expression of one or more genes in the M3 subnetwork. Modulation of expression activity may include a decrease in gene expression of one or more genes in the M3 subnetwork. Modulation of expression activity may include an increase in expression activity of one or more genes in the M3 subnetwork and a decrease in expression activity of one or more genes in the M3 subnetwork.

In one embodiment of the invention, the modulation of M3 expression activity may be for use in the treatment of a CNS trauma. The CNS trauma may include a

primary injury and/or a secondary injury. The modulation of M3 expression activity may be for the treatment of SCI. The modulation of M3 expression activity may be for the treatment of TBI. The treatment of CNS trauma may include reducing the extent of neurotraumatic injury. The treatment of CNS trauma may include promoting repair by, for example, improving blood flow, improving tissue perfusion, etc.

In one embodiment of the invention, the compound for use in modulating M3 expression activity may be a sulfonamide. The compound may be sulfaphenazole.

In one embodiment of the invention, the compound for use in modulating M3 expression activity may be formulated for delivery to a subject with CNS trauma. Methods of delivery may include intravenous, subcutaneous, intrathecal, or oral delivery.

The present disclosure is directed, at least in part, to repurposing a known drug, sulfaphenazole, for the treatment of acute traumatic SCI in order to improve functional recovery. An integrated systems biology approach was used to study gene expression in the human spinal cord, which revealed gene regulatory networks implicated in the pathophysiological response to SCI. This invention is based, at least in part, on the surprising discovery of a gene subnetwork (M3) that is enriched for genes known to be involved in the response to SCI, and whose expression is upregulated in a severity-dependent manner following injury; wherein the reversal of this expression pattern is associated with functional recovery. This invention aims at identifying chemical compounds that reverse the expression patterns of the identified expression modules to facilitate treatment of acute SCI.

Examples

The following examples are illustrative of some of the embodiments of the invention described herein. These examples do not limit the spirit or scope of the invention in any way.

Example 1: Identification and validation of literature curated genes associated with response to SCI

Systematic analysis of the literature

A systems-level analysis of the spinal cord transcriptome can be used to identify drugs with potential therapeutic activity to promote functional recovery in SCI by identifying small molecules whose effect on gene expression could reverse the aberrant expression levels observed after SCI. One method to identify drugs with this property is to first screen drugs computationally according to the tendency of a set of genes within a subnetwork to be more highly ranked in differential expression compared to randomly selected genes. To establish a set of genes implicated in the physiological response to SCI, a systematic analysis of the SCI literature, was conducted. A set of 695 unique human genes associated with the response to SCI in small-scale experiments was identified. Of these genes, 559 were upregulated following SCI and 213 were downregulated, while the protein products of 9 genes were differentially phosphorylated. 151 unique proteins were identified more than once (Figure 1A). This set represents genes that have been associated with SCI in a wide range of experimental models of SCI, in addition to human injuries (Figure 1B); in multiple species, including human as well as rat, mouse, and rabbit (Figure 1C); using a range of experimental techniques (Figure 1D); and at a variety of time points, from 1 hour to 6 months post-injury (Figure 1E).

Validation of literature-curated spinal cord injury genes

The biological relevance of the literature-curated (LC) SCI gene set was validated using multiple lines of evidence. First, it was established that LC genes were more likely to share common biological functions than random sets of genes, using annotations from the Gene Ontology (Ashburner M *et al.*, (2000) 'Gene ontology: tool for the unification of biology. The Gene Ontology Consortium' *Nat Genet* 25:25–29). Because functional annotations may be specific or broad, it was confirmed that the enrichment held regardless of the number of genes to which each term was annotated (all $P < 10^{-15}$, Figure 1C). Next, the tendency for the protein products of LC genes to physically interact was investigated. Significant enrichment for protein- protein

interactions (PPIs) between LC genes was observed relative to random expectation (empirical $P < 10^{-3}$, Figure 1D), and additionally it was established that this enrichment was not a function of the experimental technique employed for interaction detection (all $P < 10^{-3}$, Figure 2D–E). This confirmed significant enrichment for PPIs between LC genes relative to random expectation ($P < 10^{-3}$) regardless of experimental method. Genes implicated in a variety of complex diseases by genome-wide association studies (GWAS) have been found to form distinct modules of densely interacting proteins within the human interactome (Ghiassian SD, *et al.*, (2015) (*ibid*)). Hence, it was evaluated whether this same principle held for SCI by calculating the size of the largest connected component (LCC) between LC genes, and found that LC genes collectively formed a significantly larger subnetwork than random expectation (empirical $P < 10^{-5}$, Figure 1E) revealing LC genes occupy a distinct region of the human interactome ($P < 10^{-3}$). This finding was again reproduced in independent interaction datasets ($P < 10^{-5}$, Figure 2F–G) revealing LC genes occupy a distinct region of the human interactome ($P < 10^{-3}$) regardless of experimental method. LC genes also displayed a significant tendency to participate in the same protein complexes ($P < 10^{-3}$, Figure 2H). Finally, LC genes were preferentially recovered by a disease gene prediction algorithm when a subset of them were randomly withheld, and the remainder used to prioritize additional disease genes ($P < 10^{-15}$ (Kolomogorov-Smirnov test), Figure 1F, and Figure 2I). Thus, LC genes represent a biologically relevant and functionally coherent set of genes, which converge on a common protein interaction module within the human interactome.

Gene coexpression network analysis of human spinal cord

Multiple lines of evidence support the functional coherence of the set of genes implicated in SCI by small-scale experiments. However, these studies nonetheless have appreciable false positive and false negative rates, and are limited by sociological and experimental biases. Hence knowledge was integrated from the SCI corpus within an unbiased, genome-wide framework. It was hypothesized that unsupervised gene coexpression network analysis of human spinal cord would provide a powerful method to integrate these LC genes in a systems-level context, as this method has recently

been powerfully applied to develop insights into the etiologies of a number of neurological diseases (Zhang B *et al.*, (2013) 'Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease' *Cell* 153:707–720; Johnson MR *et al.*, (2015) 'Systems genetics identifies Sestrin 3 as a regulator of a proconvulsant gene network in human epileptic hippocampus' *Nat Commun* 6:6031; Delahaye-Duriez A, *et al.*, (2016) 'Rare and common epilepsies converge on a shared gene regulatory network providing opportunities for novel antiepileptic drug discovery' *Genome Biol* 17:245; Langfelder P *et al.*, (2016) 'Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice' *Nat Neurosci* 19:623–633) or neuropsychiatric diseases (Voineagu I, *et al.*, (2011) 'Transcriptomic analysis of autistic brain reveals convergent molecular pathology' *Nature* 474:380–384; Chen C, *et al.*, (2013) 'Two gene co-expression modules differentiate psychotics and controls' *Mol Psychiatry* 18:1308–1314; Fromer M *et al.*, (2016) 'Gene expression elucidates functional impact of polygenic risk for schizophrenia' *Nat Neurosci* 19:1442–1453).

Gene coexpression networks in human spinal cord was constructed using RNA-seq data from 71 post-mortem human spinal cords from the Genotype-Tissue Expression project (GTEx) (GTEx Consortium TGte (2013) 'The Genotype-Tissue Expression (GTEx) project' *Nat Genet* 45:580–585). WGCNA (Langfelder P, *et al.*, (2008) 'WGCNA: an R package for weighted correlation network analysis' *BMC Bioinformatics* 9:559) was applied to group the human spinal cord transcriptome into 15 distinct modules of coexpressed genes. These modules represent networks of genes that share highly related patterns of expression in the human spinal cord. In order to establish the reproducibility of these spinal cord gene expression modules in an independent dataset, a second human spinal cord gene coexpression network was constructed from public microarray data, using established techniques to control for batch effects (Leek JT, *et al.*, (2012) 'The sva package for removing batch effects and other unwanted variation in high-throughput experiments' *Bioinformatics* 28:882–883).

The reproducibility of these spinal cord gene coexpression modules was evaluated using the Z_{summary} value, developed by Langfelder *et al.*, The authors suggest that a Z_{summary} value < 2 indicates no evidence for module preservation in a second

dataset, whereas $Z_{\text{summary}} > 10$ reflects strong evidence for module preservation; values between 2 and 10 are indicative of weak to moderate preservation. Despite the small sample size of the microarray coexpression network ($n = 34$), seven of 15 modules showed strong evidence of reproducibility ($Z_{\text{summary}} > 10$), with an additional two modules showing moderate evidence of reproducibility ($Z_{\text{summary}} > 5$) (Figure 2A). Only two of 15 modules showed no evidence of reproducibility ($Z_{\text{summary}} < 2$).

Next, the evolutionary conservation of human spinal cord coexpression modules was investigated in mouse and rat, two of the most commonly used model organisms for studies of SCI pathophysiology. Hundreds of microarray samples of mouse ($n = 415$) and rat ($n = 268$) spinal cords from the Gene Expression Omnibus were compiled, and mouse and rat spinal cord gene coexpression networks were constructed. Five modules showed strong evidence of conservation ($Z_{\text{summary}} > 10$) in both species, while another four modules showed moderate evidence of conservation ($Z_{\text{summary}} > 5$) in at least one species, and only two modules showed no evidence of conservation in either species ($Z_{\text{summary}} < 2$) (Figure 2A). Notably, the same five modules that showed the strongest evidence of reproducibility (M2, M3, M7, M8, and M12) also showed the strongest evidence of conservation in rat and mouse.

In order to integrate the LC gene set with the spinal cord coexpression network, enrichment of LC genes within each module was tested for (Figure 3B). Two modules, M3 and M7, were significantly enriched for LC genes (Fisher's exact test, Bonferroni-corrected $P = 3.8 \times 10^{-8}$ and 2.0×10^{-3} , respectively). These modules consist of 746 and 330 genes, respectively, and both are among the most reproducible and conserved in the spinal cord (Figure 3A). The robustness of the observed enrichment was confirmed by randomly removing seed genes from the LC set, and by randomly adding false positive genes to the LC set. Both M3 and M7 remained significantly enriched for LC genes despite the removal of a large number of seed genes, or the addition of a large number of random genes (Figure 3C): M3 remained significantly enriched for LC genes even after the removal of approximately 70% of genes from the seed set, compared to approximately 50% for M7. Moreover, M3 remained significantly enriched for seed

genes even after the size of the literature-curated set was doubled by addition of random false positives.

Finally, to assess the relationships between modules, a module meta-network based on the eigengene of each module was constructed, defined as the first principal component of module expression (Figure 3D) (Langfelder P, *et al.*, (2007) 'Eigengene networks for studying the relationships between co-expression modules' *BMC Syst Biol* 1:54). In the resulting network, M3 and M7 clustered together, as would be expected given the strong correlation between their eigengenes (Spearman's $\rho = 0.54$, $P = 1.6 \times 10^{-6}$). These results suggest that the expression of these two modules in the spinal cord is highly correlated.

In summary, gene coexpression network analysis identified five highly conserved and reproducible modules, two of which are significantly and robustly enriched for LC genes, and whose expression is highly correlated.

Regulation of spinal cord coexpression modules in spinal cord injury

The roles of M3 and M7, as well as other highly conserved coexpression modules, were characterized in the pathophysiological response to SCI. A meta-analysis of five mouse and rat transcriptomic studies of SCI within the context of the spinal cord coexpression network was performed in order to identify consensus changes in the spinal cord transcriptome at the module level in response to SCI (Figure 3E). This analysis identified M3, M6, M7, and M11 as consensus upregulated, and M1 and M2 as consensus downregulated, following SCI. One other module, M8, was upregulated following SCI in four of five datasets, while the remaining eight modules did not show robust evidence of differential expression. Among all seven modules, M2, M3, and M7 consistently showed the strongest evidence of differential expression (Figure 3F, $P \leq 1.2 \times 10^{-78}$, 2.0×10^{-55} , and 1.7×10^{-14} , respectively). Notably, among these modules, M2, M3, M7 were strongly conserved and reproducible in mouse, rat, and human networks ($Z_{\text{summary}} > 10$), whereas M1, M6, and M11 displayed only moderate evidence of conservation ($2 < Z_{\text{summary}} < 10$), suggesting these modules may capture

human- specific aspects of spinal cord transcriptome organization that are relevant in the response to SCI.

Because the pathophysiological processes underlying primary and secondary injury in SCI are incompletely understood, the expression of spinal cord modules at acute, subacute, and chronic time points was additionally investigated. Consensus module expression was remarkably consistent at all timepoints studied (Figure 3G). However, analysis of the temporal regulation of spinal cord modules revealed consensus downregulation of M9 at the most acute time point after SCI, but consensus upregulation at a chronic time point. These results suggest M9 may be specifically involved in the transition between acute and chronic physiological responses following SCI. Thus, by integrating gene coexpression network analysis with a meta-analysis of the SCI transcriptome, a consensus network signature associated with the response to SCI was revealed, and a network module specifically implicated in the transition from acute to chronic injury processes.

Example 2: Functional characterization of spinal cord modules

The biological significance of the modules implicated in the physiological response to SCI was characterized by integrating functional annotations from the Gene Ontology (Ashburner *et al.*, (*ibid*)) and molecular signatures from MSigDB (Liberzon A, *et al.*, (2011) 'Molecular signatures database (MSigDB) 3.0' *Bioinformatics* 27:1739–1740). To visualize statistically overrepresented gene sets, enrichment maps for each consensus signature module were constructed (Merico D, *et al.*, (2010) 'Enrichment map: a network-based method for gene-set enrichment visualization and interpretation' Ravasi T, ed. *PLoS One* 5:e13984) (Figure 4A–B). To appreciate the cell type-specificity of each module, a meta-analysis of transcriptomic and proteomic profiles from the major cell types of the CNS was constructed (Figure 4C) (Cahoy JD, *et al.*, (2008) 'A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function' *J Neurosci* 28:264–278; Zhang Y, *et al.*, (2014) 'An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex' *J Neurosci* 34:11929–11947; Sharma K, *et al.*, (2015) 'Cell type- and brain region-resolved mouse brain proteome' *Nat Neurosci*

18:1819–1831). M1 was an oligodendrocyte module, associated with axon ensheathment and myelination, whereas M2 was a neuronal module implicated in synaptic transmission. M3 was enriched for markers of microglia and vascular endothelial cells, and biological processes such as inflammatory response and response to wounding, while M7 was a microglial module enriched for annotations related to the immune response. M9 was enriched for astrocyte markers and terms such as oxidation-reduction process, as well as the term central nervous system development, which may be related to its upregulation at chronic time points following SCI. M6 and M11 were not significantly associated with any specific cell type, and were enriched for terms including cellular protein modification process and mitochondrial translation, respectively.

Example 3: Relationship of spinal cord modules to SCI severity and recovery

The finding that M3 is a highly conserved and reproducible gene coexpression module, with the most significant enrichment for LC genes and strong evidence of upregulation following SCI, suggested that this module plays a key pathophysiological role in SCI. The role of M3 in SCI was investigated by focusing on the relationship between M3 expression and two key clinical parameters in SCI: injury severity and recovery of sensory and motor function.

First, gene expression data from a mouse model of severity-dependent injury was re-analyzed to identify relationships between consensus module expression and injury severity (Di Giovanni S, *et al.*, (2003) 'Gene profiling in spinal cord injury shows role of cell cycle in neuronal death' *Ann Neurol* 53:454–468; De Biase A, *et al.*, (2005) 'Gene expression profiling of experimental traumatic spinal cord injury as a function of distance from impact site and injury severity' *Physiol Genomics* 22:368–381). Strikingly, M3 was the sole module enriched for genes positively correlated to injury severity, whereas M1, M2, and M9 were enriched for genes anti-correlated to injury severity (Figure 5A). This effect was investigated further by considering the correlations between module eigengenes, which provide a summary of the expression profile of each module, and injury severity. This analysis revealed that the M3 eigengene was the most strongly correlated with injury severity (Spearman's $\rho = 0.65$, $P = 1.6 \times 10^{-19}$), with a clear

separation in M3 expression between the mild, severe, and sham injury groups at 7 days post-injury (Figure 5E).

In order to validate the severity-dependent upregulation of M3 following SCI, a prospective experimental SCI study was conducted, using the field standard contusion injury model at the T10 segment, and RNA sequencing of the spinal cord parenchyma in rats subjected to moderate, severe, or sham injuries ($n = 5$ per group) was performed. A novel RNA-seq data reproduced the consensus network signature derived from our meta-analysis of microarray datasets, emphasizing the robustness of this systems-level characterization of SCI pathophysiology (Figure 5F). In addition, the significant association between injury severity and the M3 eigengene was confirmed (Figure 5E; Spearman's $\rho = 0.94$, $P = 4.2 \times 10^{-7}$). Thus, insights into the network-level organization of the transcriptome in SCI derived from a meta-analysis of publicly available data replicate in an independently collected dataset.

Together, these results emphasized the severity-dependent upregulation of M3 following SCI, and suggested that the expression of a gene or combination of genes that accurately summarize the transcriptional status of M3 has the potential to serve as an objective biomarker of SCI severity. To evaluate the potential of such an indicator as a biomarker of injury severity, the hub genes of M3 were further investigated. These genes are the most central and interconnected within the module, based on their correlation to the module eigengene, and are highly enriched for functionally relevant genes such as drivers of disease pathophysiology (Voineagu *et al.*, 2011 (*ibid*)) or therapeutic targets (Horvath S *et al.*, (2006) 'Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target' *Proc Natl Acad Sci U S A* 103:17402–17407). Consistent with these findings, the hubness of M3 genes (that is, their correlation to the M3 eigengene in human spinal cord) was significantly associated with their predictive power as a biomarker of injury severity (Figure 5D; Spearman's $\rho = 0.23$, $P = 3.9 \times 10^{-7}$). Among M3 hubs, six genes stratified rats by SCI severity with an accuracy greater than 90%, including ANXA1, COLGALT1, IFNGR2, SHC1, SOD2, and TBC1D2B (Figure 5G). Remarkably, expression levels of ANXA1 (annexin A1) stratified moderately and severely injured rats with perfect accuracy (Figure 5I). Annexin A1 has previously been associated with SCI by three small-scale studies, each employing

divergent model organisms, spinal cord levels, and injury models, emphasizing the robustness of the association between SCI and annexin upregulation (Gao Q, *et al.*, (2012) 'Differential protein expression in spinal cord tissue of a rabbit model of spinal cord ischemia/reperfusion injury' *Neural Regen Res* 7:1534–1539; Didangelos A, *et al.*, (2016) 'High-throughput proteomics reveal alarmins as amplifiers of tissue pathology and inflammation after spinal cord injury' *Sci Rep* 6:21607; Moghieb A, *et al.*, (2016) 'Differential Neuroproteomic and Systems Biology Analysis of Spinal Cord Injury' *Mol Cell Proteomics* 15:2379–2395).

While the integrative analyses of public and newly acquired transcriptomic data established a strong relationship between M3 expression and SCI severity, post-transcriptional regulation can result in marked differences between gene and protein expression levels, particularly in complex tissues such as those of the CNS (Sharma *et al.*, 2015 (*ibid*)). To further explore the potential of M3 hubs as biomarkers of SCI severity, quantitative proteomic profiling of the same rat spinal cords was performed. The overall structure of the spinal cord coexpression network was conserved between the transcriptomic and proteomic levels. Despite having limited power to detect module preservation due to the small size of the proteomic sample ($n = 15$), both M3 and M7 displayed highly significant evidence of reproducibility between the RNA and protein levels (Figure 5C; $Z_{\text{summary}} = 6.8$ and 7.3 , respectively). Furthermore, substantial overall agreement between proteomic data and the consensus network signature derived from transcriptomic meta-analysis, further validated the robustness of the systems-level portrait of SCI pathophysiology (Figure 5F). Finally, the severity-dependent upregulation of both the M3 eigengene and annexin A1 in particular (Figure 5H and Figure 5J), finding that AnxA1 protein levels stratified both moderate and severe injuries was confirmed with an accuracy of 93%. Thus, systems-level insights into SCI pathophysiology derived from integrative transcriptomic analyses extend to the proteomic level and nominate quantitative biomarkers of SCI severity.

Given the strong relationship between injury severity and M3 expression, it was hypothesized that targeting the transcriptional profile of this module could represent a viable strategy for development of novel therapies for SCI. To explore this hypothesis, gene expression data from a recent trial of a neurotrophic factor, neurotrophin-3 (NT-3),

which promoted sensory and motor recovery after SCI was analyzed (Duan H, *et al.*, (2015) 'Transcriptome analyses reveal molecular mechanisms underlying functional recovery after spinal cord injury' *Proc Natl Acad Sci U S A* 112:13360–13365; Yang Z, *et al.*, (2015) NT3-chitosan elicits robust endogenous neurogenesis to enable functional recovery after spinal cord injury. *Proc Natl Acad Sci U S A* 112:13354–13359).

Remarkably, all six consensus modules derived from the meta-analysis, including M3, were differentially expressed at the lesion site in the opposite direction (Figure 5F) in rats treated with NT-3. Intriguingly, the sole other differentially expressed module was M9, which was previously observed to exhibit a strongly time-dependent expression profile, and which was enriched for genes associated with neurogenesis. In rats treated with NT-3, known for its role in neuronal differentiation, axonal growth, and chemotropic guidance (Alto LT, *et al.*, (2009) 'Chemotropic guidance facilitates axonal regeneration and synapse formation after spinal cord injury' *Nat Neurosci* 12:1106–1113; Anderson MA, *et al.*, (2016) 'Astrocyte scar formation aids central nervous system axon regeneration' *Nature* 532:195–200), M9 was strongly upregulated at the lesion site relative to the experimental control ($P = 7.4 \times 10^{-6}$). Moreover, the M3 eigengene was significantly downregulated in NT-3-treated rats relative to controls (Figure 5K; one-tailed Wilcoxon rank-sum test, $P = 2.0 \times 10^{-3}$). These results indicate that reversal of the transcriptome changes observed in response to SCI is associated with functional recovery in a rat model, and highlight M3 expression as a predictor of functional recovery.

Example 4: Identification of drug entities that target M3

An integrated systems biology approach identified a gene regulatory subnetwork ("M3") consisting of 746 genes, that was enriched for genes associated with SCI by small-scale experiments, was reproducible in a second human dataset, was highly conserved in rat and mouse, and was associated with the inflammatory response and microglial and vascular epithelial cells in the spinal cord. This module is upregulated following SCI in a severity-dependent manner and downregulated in treatments that promote functional recovery. To identify compounds that could reverse the expression

patterns of M3 and potentially reverse the aberrant expression levels observed after SCI, drugs were computationally screened according to the tendency of a set of genes within a subnetwork to be more highly ranked in differential expression compared to randomly selected genes following drug exposure. Currently, the largest database to report transcriptome-wide expression following drug exposure is the Connectivity Map (CMap), which provides the signatures for 1300 compounds (Lamb J *et al.*, (2006) 'The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease' *Science* (80-) 313:1929–1935). To cause downregulation of M3, sulfaphenazole was most highly ranked ($P = 1.5 \times 10^{-23}$) of M3 in the CMap database. A similar study was carried out using M9 in order to evaluate traumatic brain injury, which again revealed sulfaphenazole as most highly ranked in the CMap database. Sulfaphenazole is a specific inhibitor of CYP2C9. It blocks pro-inflammatory and atherogenic effects of linoleic acid (increase in oxidative stress and activation of AP-1) mediated by CYP2C9, and inhibits bradykinin-induced tPA release. These effects may serve to increase blood flow and prevent aberrant vasoconstriction in response to inflammation, which could have direct and critical links to the pathophysiology of SCI.

Example 5

A meta-analysis of hundreds of published microarray and RNA-seq samples in human, rat, and mouse spinal cords, and sequenced spinal cords across injury severities was conducted, to comprehensively investigate the effects of SCI on the spinal cord transcriptome (Squair *et al.*, (2018), 'Integrated systems analysis reveals conserved gene networks underlying response to spinal cord injury', *Elife* 2;7. pii: e39188. doi: 10.7554/eLife.39188). A systems biology analysis revealed a coexpressed subnetwork of genes that is upregulated in a severity-dependent manner following injury, and selectively downregulated in conditions that promote functional recovery (Figure 6). This gene subnetwork is reproducible across human datasets, evolutionarily conserved in rodents, and highly expressed in microglia and vascular endothelial cells. Compounds that downregulate the expression of this gene subnetwork are thought to be useful for treatment of SCI.

An established computational biology approach was applied to rank known drugs on the basis of their ability to reverse the upregulation of this severity-dependent gene subnetwork in SCI as candidates for use as drugs for treatment of SCI (Figure 7A). A similar approach was applied to rank known drugs on the basis of their ability to reverse the upregulation of a gene subnetwork ("M9") upregulated in the brain cortex in TBI (Figure 7B). The top-ranked candidate, sulfaphenazole, is a sulfonamide antibiotic that has previously shown efficacy for treatment of ischemia-reperfusion injury and wound healing disorders (Figure 7).

To test the *in vivo* efficacy of sulfaphenazole two preliminary studies were conducted. In study 1, sulfaphenazole was tested to determine whether it reverses the microglia subnetwork signature using RNA sequencing in rodents subjected to the field standard contusion SCI at the T10 spinal level. In study 1, 15 rats were evenly assigned to a control group, a SCI group treated with saline, or a SCI group treated with sulfaphenazole every 12 hours. In study 2, sulfaphenazole was tested to determine whether it was able to improve cardiovascular function in the previously validated high-thoracic (T3 spinal level) contusion model of SCI (Squair JW, *et al.*, (2017 - Epub 2016 Aug 25) 'High Thoracic Contusion Model for the Investigation of Cardiovascular Function after Spinal Cord Injury', *J Neurotrauma*. 34(3):671-684. doi: 10.1089/neu.2016.4518.; Squair JW, *et al.*, (2018 - Epub 2017 Oct 13), 'Spinal Cord Injury Causes Systolic Dysfunction and Cardiomyocyte Atrophy', *J Neurotrauma*, 35(3):424-434. doi: 10.1089/neu.2017.4984; Squair JW, *et al.*, (2018 - Epub 2018 Jan 9) 'Spinal cord injury-induced cardiomyocyte atrophy and impaired cardiac function are severity dependent', *Exp Physiol.*, 103(2):179-189. doi: 10.1113/EP086549) In study 2, 15 rats were evenly assigned to a control group, a SCI group treated with saline, or a SCI group treated with sulfaphenazole every 12 hours.

Findings from study 1 are summarized in Figure 8. Figures 8A, 10 and 11 illustrate findings from M3 and SCI, while Figure 8B illustrates findings from M9 and TBI. Rats treated with sulfaphenazole were found to have a transcriptomic signature indicating the reversal of expression in key gene subnetworks. Importantly, the transcriptomic signature identified in the rats treated with sulfaphenazole is almost

identical to a previous study in the field of SCI (that studied NT-3) that reported their treatment ultimately resulted in improved neurological outcomes.

Findings from study 2 are summarized in Figure 9 (Note the taller and wider pressure-volume loops in SP vs. SCI and that SP improved all induces towards sham-injured animals, implying a reversal/prevention of cardiovascular dysfunction post-SCI). Rats treated with sulfaphenazole (sp) exhibited a larger and wider left-ventricular pressure volume loop, that when subjected to inferior vena cava occlusions (which causes the leftward shift in the pressure-volume loop) resulted in a steeper slope of the end-systolic pressure volume relationship implying improved end-systolic elastance (E_{es}), which is the gold-standard measure of cardiac function *in vivo*. There were additional increases in both resting systolic blood pressure as well as the maximal rate of pressure change inside the left-ventricle (dP/dT_{max}).

Overall, the experiments and results described herein provide *in vivo* evidence that sulfaphenazole is efficacious in both reversing the expression profile of key gene subnetworks implicated in the response to SCI as well as in improving function of the cardiovascular system post-SCI. The results also point to the effectiveness of the approaches described herein, including, but not limited to, repurposing a rationally identified drug to target multiple systems and suggests that this latter approach has the potential to significantly accelerate pharmacotherapy development for acute SCI.

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. Furthermore, numeric ranges are provided so that the range of values is recited in addition to the individual values within the recited range being specifically recited in the absence of the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the

phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Furthermore, material appearing in the background section of the specification is not an admission that such material is prior art to the invention. Any priority document(s) are incorporated herein by reference as if each individual priority document were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

What is claimed is:

1. Use of a sulfonamide for treatment of central nervous system trauma.
2. The use of claim 1 wherein the sulfonamide is selected from the group consisting of: Acetazolamide, Acetohexamide, Amprenavir, Apricoxib, Asunaprevir, Azabon, Beclabuvir, Bosentan, Brinzolamide, Bumetanide, Carbutamide, Celecoxib, Chlorpropamide, Chlorthalidone, Clopamide, Darunavir, Dasabuvir, Delavirdine, Dofetilide, Dorzolamide, Dronedarone, Ethoxzolamide, Fosamprenavir, Furosemide, Glibenclamide, Glibornuride, Gliclazide, Glyclopamide, Glimepiride, Glipizide, Gliquidone, Glisoxepide, Grazoprevir, Hydrochlorothiazide, Ibutilide, Indapamide, Mafenide, Mefruside, Methazolamide, Metolazone, Parecoxib, Paritaprevir, Probenecid, Simeprevir, Sotalol, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfametopyrazine, Sulfametoxydiazine, Sulfamoxole, Sulfanitran, Sulfaphenazole, Sulfasalazine, Sulfisomidine, Sultiame, Sumatriptan, Tamsulosin, Terephtyl, Tipranavir, Tolazamide, Tolbutamide, Udenafil, Xipamide, and Zonisamide.
3. The use of claim 1 or 2 wherein the sulfonamide is sulfaphenazole.
4. The use of any one of claims 1 to 3 wherein the central nervous system trauma is a spinal cord injury.
5. The use of any one of claims 1 to 3 wherein the central nervous system trauma is a traumatic brain injury.
6. The use of any one of claims 1 to 5 wherein the central nervous system trauma is a primary injury or a secondary injury.
7. A method of identifying a compound suitable for treatment of central nervous system trauma, the method comprising:

(a) providing a subject having central nervous system trauma wherein a severity-dependent gene subnetwork is upregulated in the subject;

(b) administering a test compound to the subject; and

(c) determining whether the upregulation of the severity-dependent gene subnetwork is reversed in the presence of the of the test compound, wherein the reversal of the upregulation is in indication that the test compound is suitable for treatment of central nervous system trauma.

8. The method of claim 7 wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is selected from the group consisting of: M1, M2, M3, M6, M7, M8, and M11.

9. The method of claim 7 or 8 wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is M3 or M7.

10. The method of any one of claims 7 to 9 wherein the severity-dependent gene subnetwork is a spinal cord gene subnetwork and is M3.

11. The method of any one of claims 7 to 10 wherein the central nervous system trauma is spinal cord injury.

12. The method of claim 7 wherein the severity-dependent gene subnetwork is a brain cortex gene subnetwork and is M9.

13. The method claim 7 or 12 wherein the central nervous system trauma is a traumatic brain injury.

14. The method of any one of claims 7 to 13 wherein the central nervous system trauma is a primary injury or a secondary injury.

15. Use of a compound identified using the method of any one of claims 7 to 14 for treatment of central nervous system trauma.
16. A method of medical treatment comprising administering a compound identified using the method of any one of claims 7 to 15 to a patient having central nervous system trauma.
17. A method of medical treatment comprising administering a sulfonamide to a patient having central nervous system trauma.
18. The method of claim 17 wherein the sulfonamide is selected from the group consisting of: Acetazolamide, Acetohexamide, Amprenavir, Apricoxib, Asunaprevir, Azabon, Beclabuvir, Bosentan, Brinzolamide, Bumetanide, Carbutamide, Celecoxib, Chlorpropamide, Chlorthalidone, Clopamide, Darunavir, Dasabuvir, Delavirdine, Dofetilide, Dorzolamide, Dronedarone, Ethoxzolamide, Fosamprenavir, Furosemide, Glibenclamide, Glibornuride, Gliclazide, Glycropyramide, Glimepiride, Glipizide, Gliquidone, Glisoxepide, Grazoprevir, Hydrochlorothiazide, Ibutilide, Indapamide, Mafenide, Mefruside, Methazolamide, Metolazone, Parecoxib, Paritaprevir, Probenecid, Simeprevir, Sotalol, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfametopyrazine, Sulfametoxydiazine, Sulfamoxole, Sulfanitran, Sulfaphenazole, Sulfasalazine, Sulfisomidine, Sultiame, Sumatriptan, Tamsulosin, Terephtyl, Tipranavir, Tolazamide, Tolbutamide, Udenafil, Xipamide, and Zonisamide.
19. The method of claim 17 or 18 wherein the sulfonamide is sulfaphenazole.
20. The method of any one of claims 17 to 19 wherein the central nervous system trauma is a spinal cord injury.
21. The method of any one of claims 17 to 19 wherein the central nervous system trauma is a traumatic brain injury.

22. The method of any one of claims 17 to 21 wherein the central nervous system trauma is a primary injury or a secondary injury.

FIG. 1

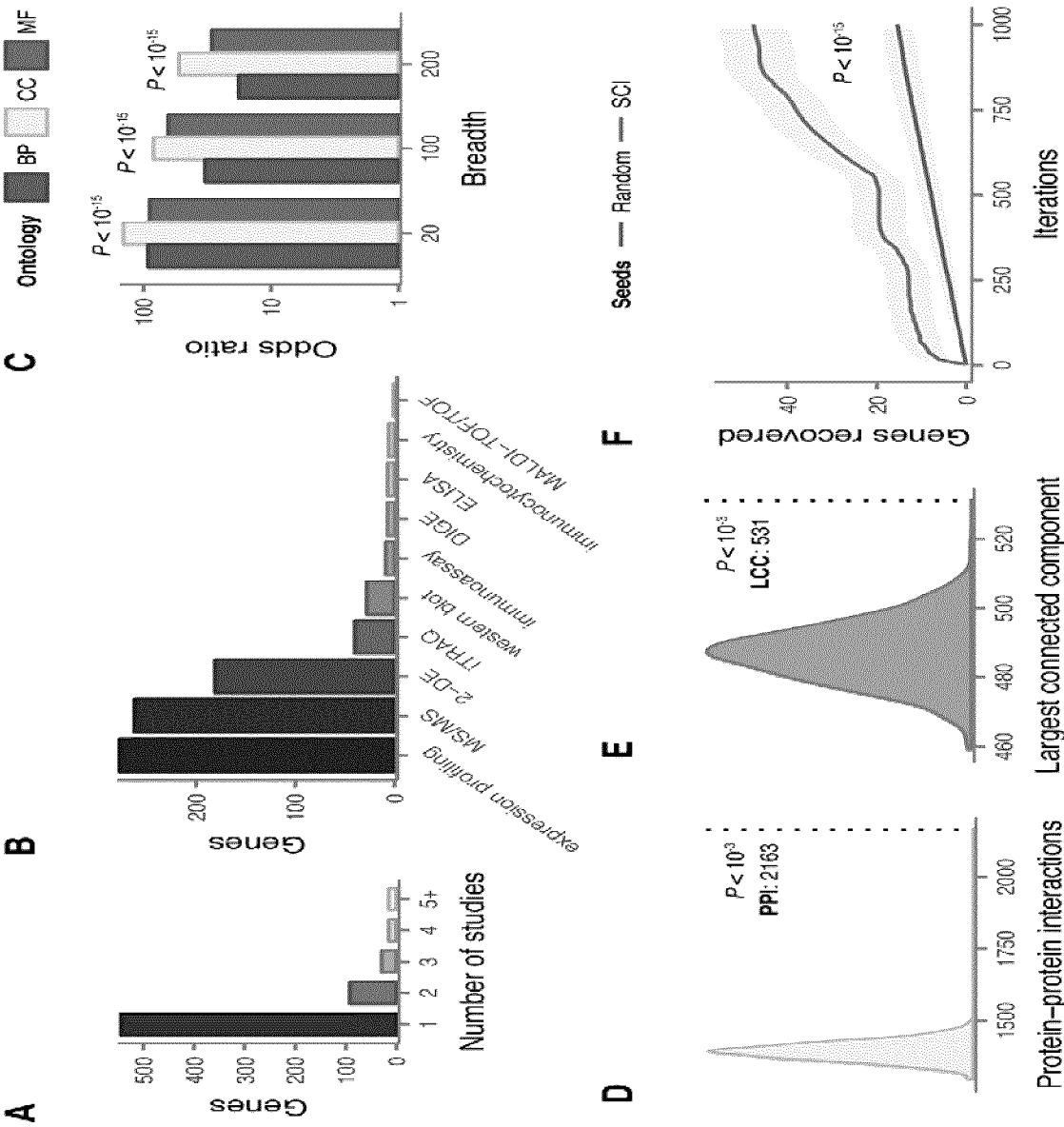


FIG. 2

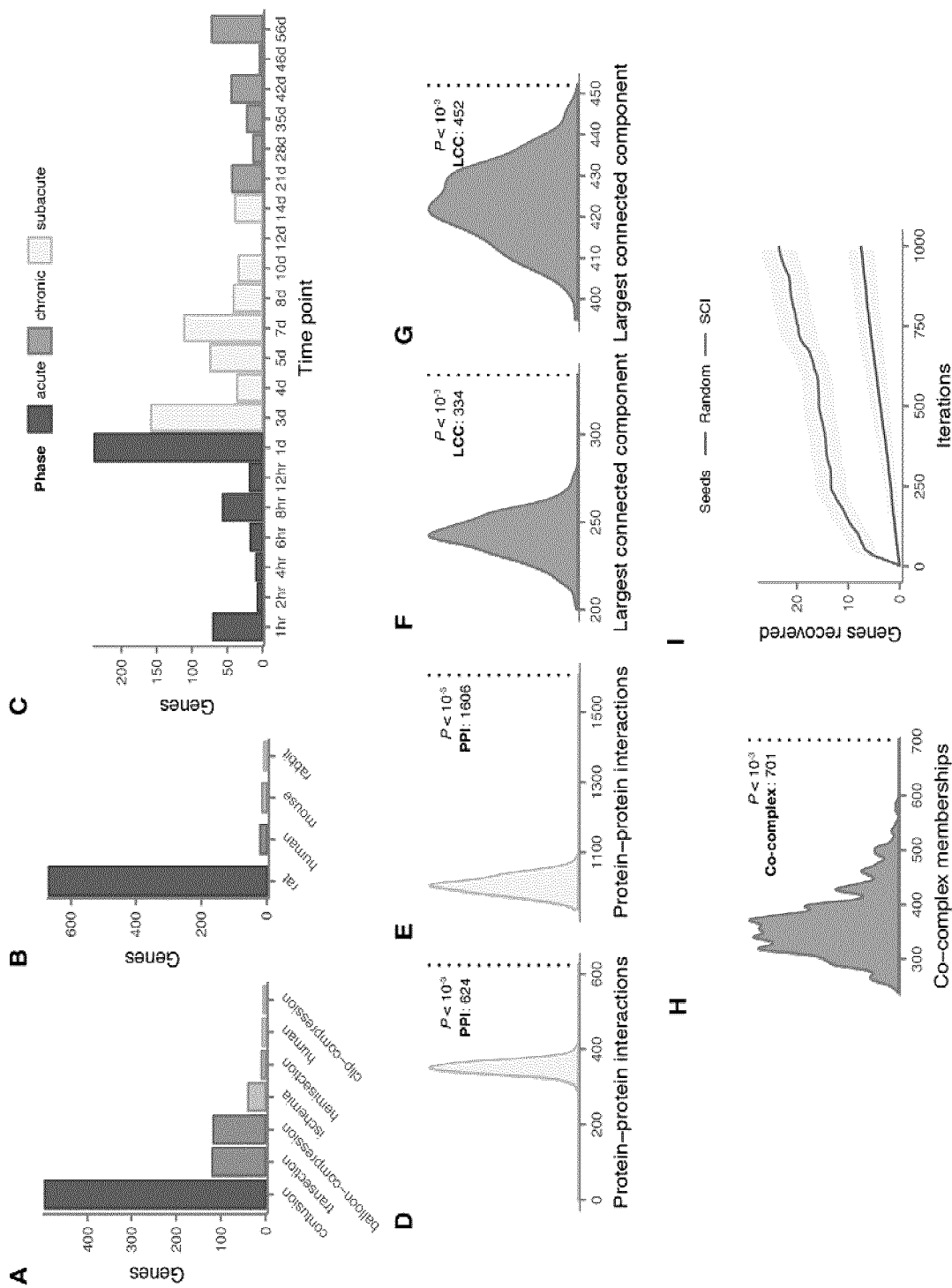


FIG. 3

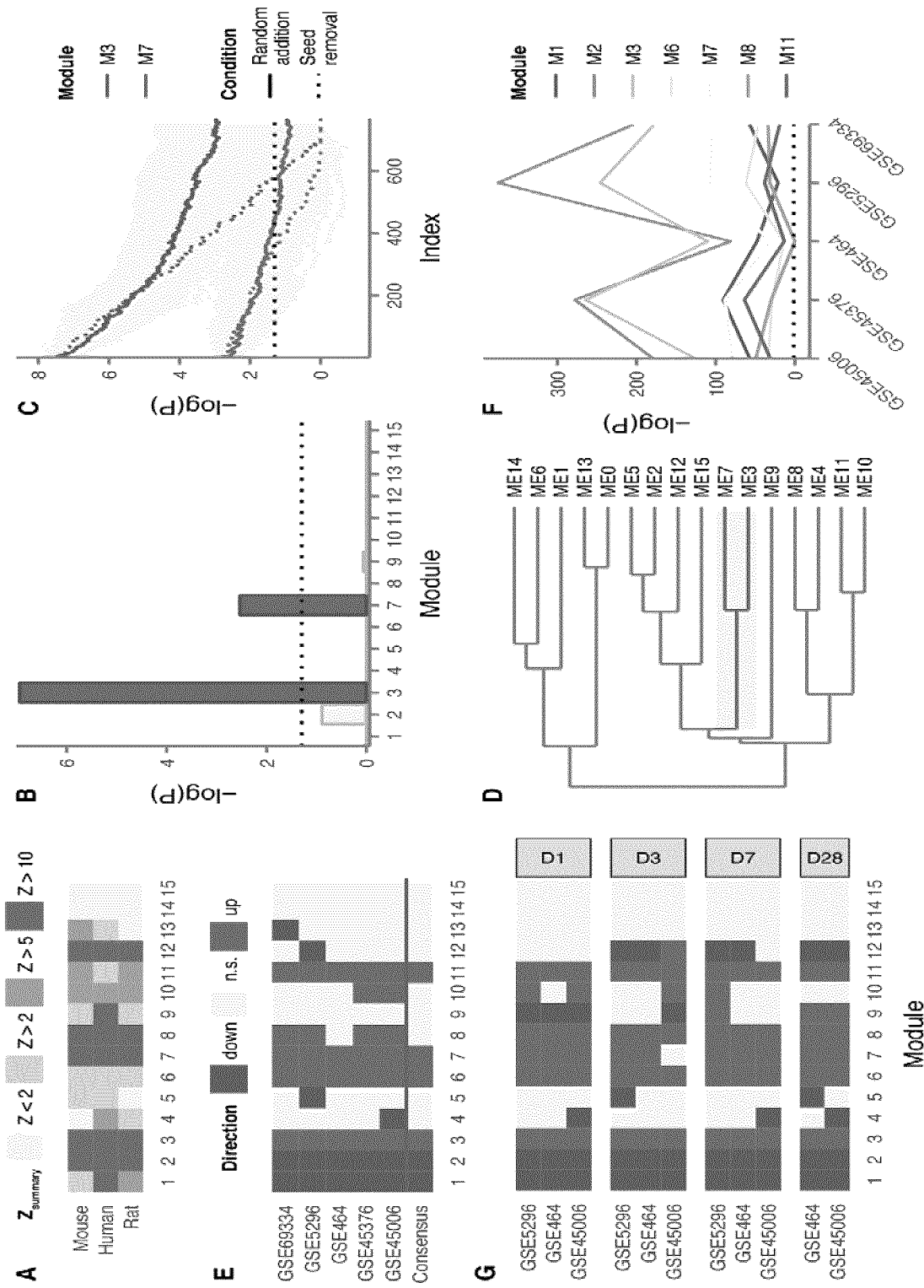


FIG. 4

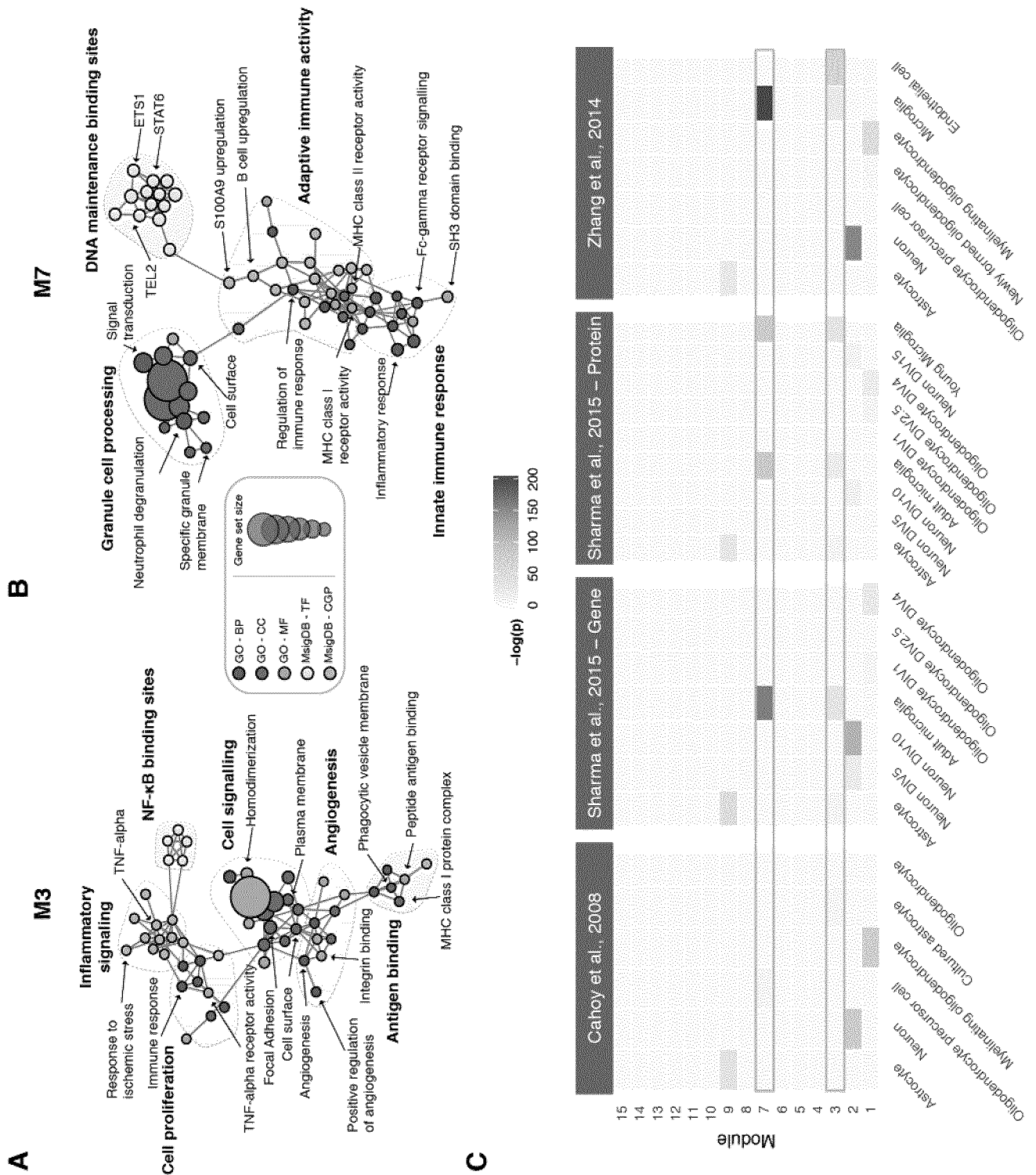


FIG. 5

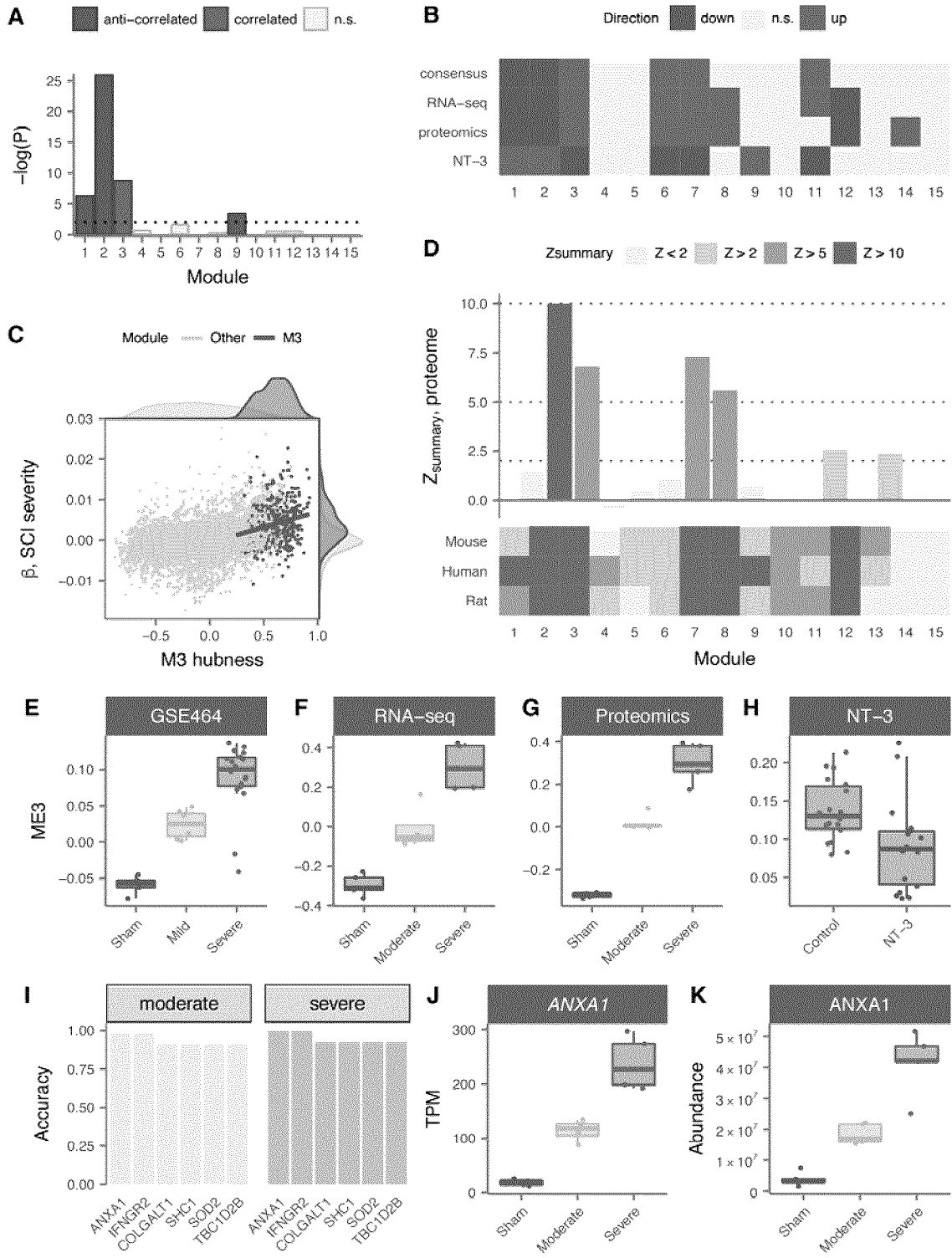
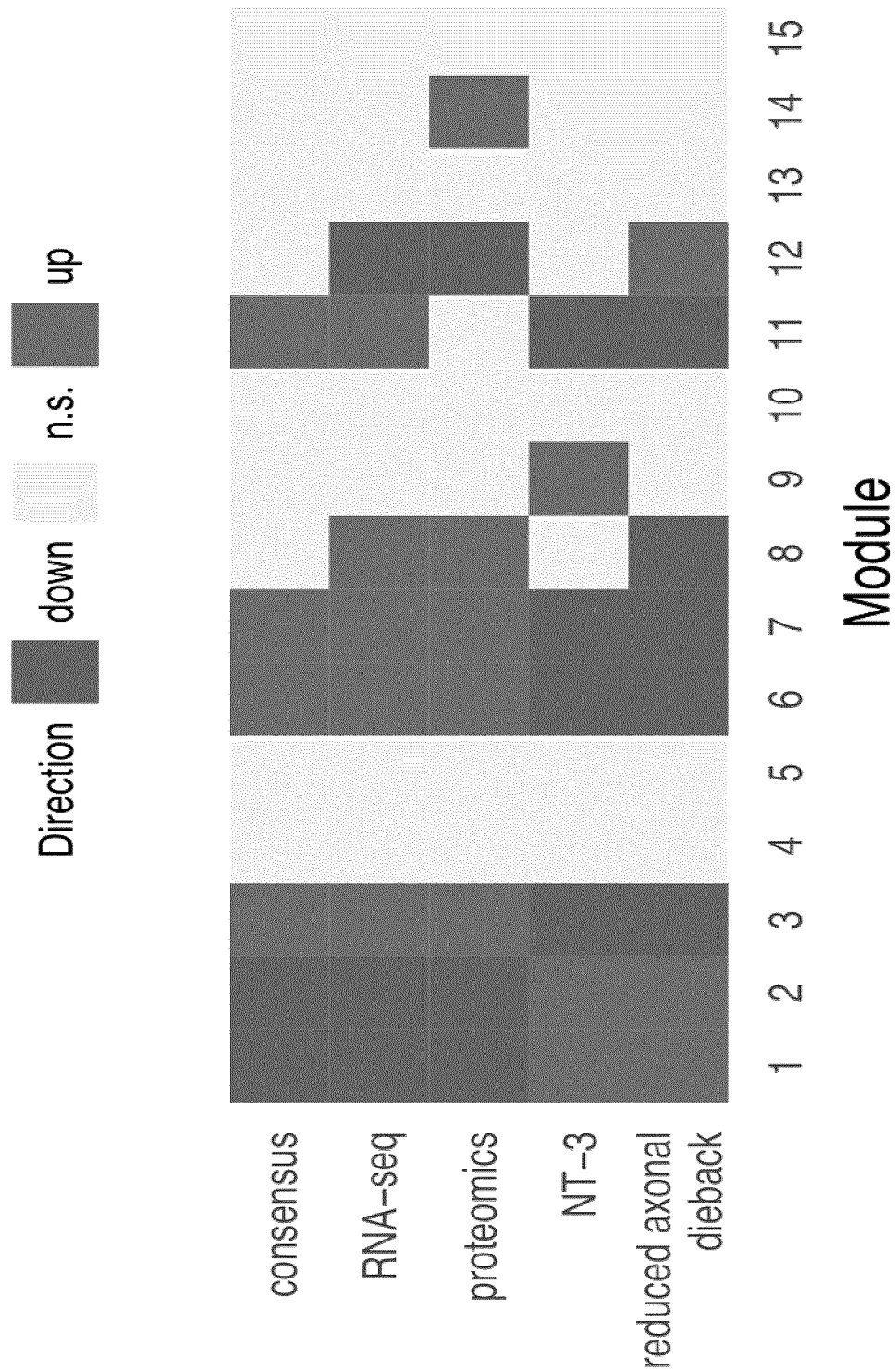


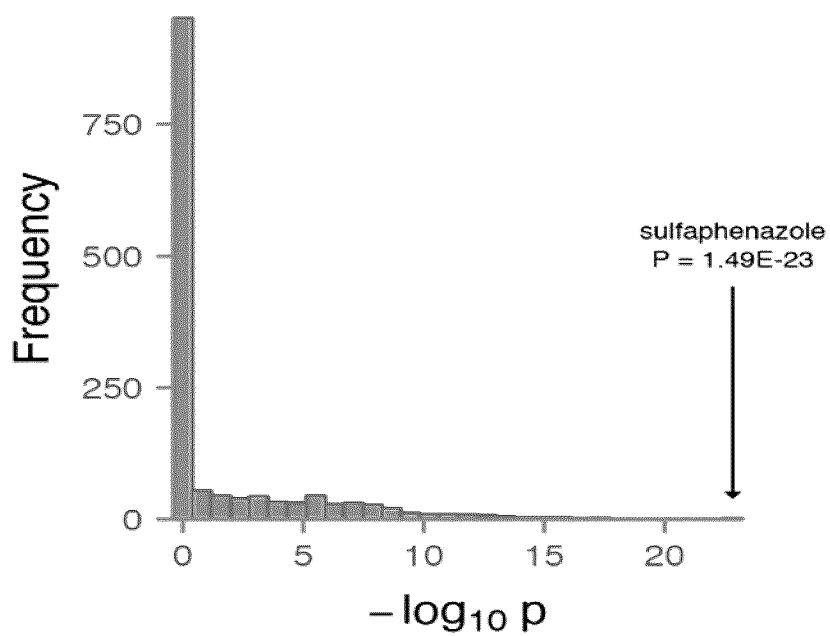
FIG. 6



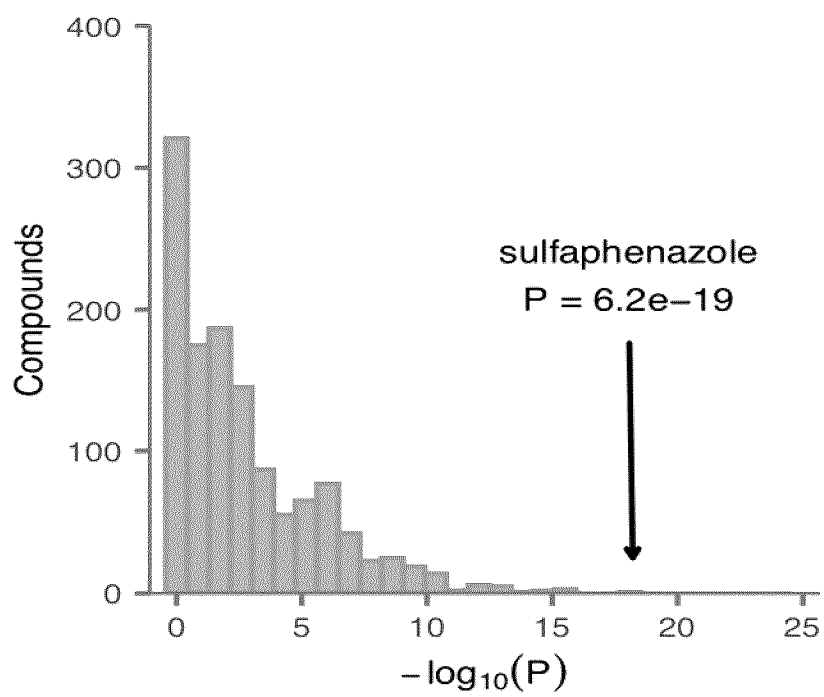
7/11

FIG. 7

A)



B)



Direction

- down
- n.s.
- up

Dataset	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6	Module 7	Module 8	Module 9	Module 10	Module 11	Module 12	Module 13	Module 14	Module 15
Meta-analysis consensus (eLife 2018)	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	n.s.	n.s.	n.s.	n.s.
NT-3	down	down	down	n.s.	n.s.	down	down	n.s.	down	n.s.	down	n.s.	n.s.	n.s.	n.s.
reduced axonal dieback	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	down	n.s.	n.s.	n.s.
RNA-seq, severity (eLife 2018)	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	down	n.s.	n.s.	n.s.
Proteomics, severity (eLife 2018)	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	down	n.s.	down	n.s.
RNA-seq, SCI vs. control (2019)	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	down	n.s.	down	n.s.
Proteomics, SCI vs. control (2019)	down	down	down	n.s.	n.s.	down	down	n.s.	n.s.	n.s.	down	down	n.s.	down	n.s.
RNA-seq, sulfaphenazole (2019)	down	down	down	n.s.	down	down	down	n.s.	down	n.s.	down	down	n.s.	n.s.	n.s.
Proteomics, sulfaphenazole (2019)	n.s.	down	down	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	down	n.s.

Module

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Direction: down (dark gray), n.s. (light gray), up (medium gray)

Module	1	2	3	4	5	6	7	9	11	13	15	17	18	21	22	24	26	31	33
Allen	down	n.s.	down	down	down	down	down	down	n.s.	down	n.s.	down	n.s.	down	n.s.	down	n.s.	down	down
GSE80174	down	down	down	down	down	down	down	down	down	n.s.	down	down	down	down	down	down	down	down	down
GSE58485	n.s.	down	down	n.s.	down	n.s.	n.s.	down	down	down	down	n.s.	down	down	down	down	down	down	down
GSE24047	down	down	down	n.s.	n.s.	n.s.	down	down	down	down	down	down	down	down	n.s.	down	down	down	down
Consensus	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	down	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Module (Tissue = cortex)

FIG. 9

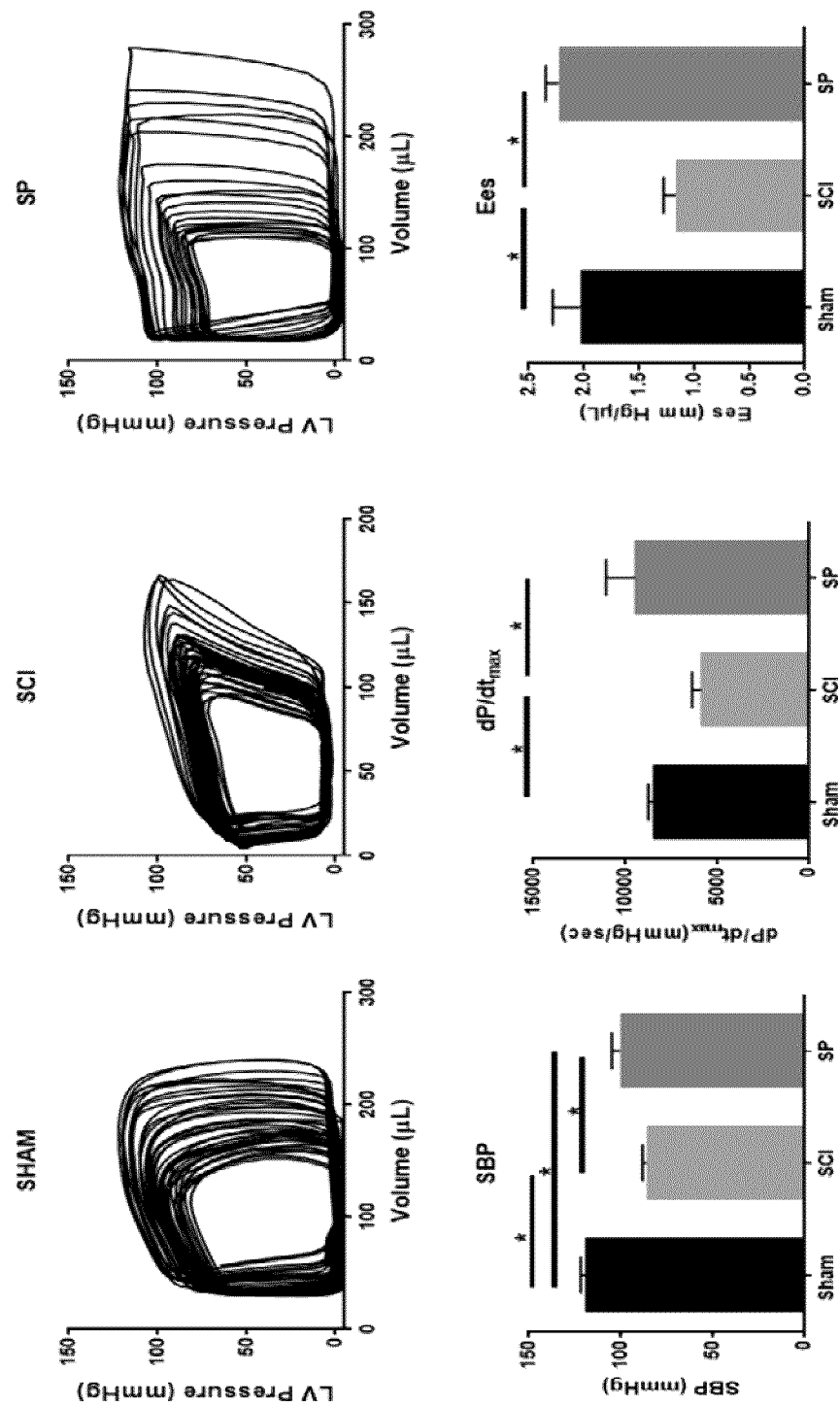


FIG. 10

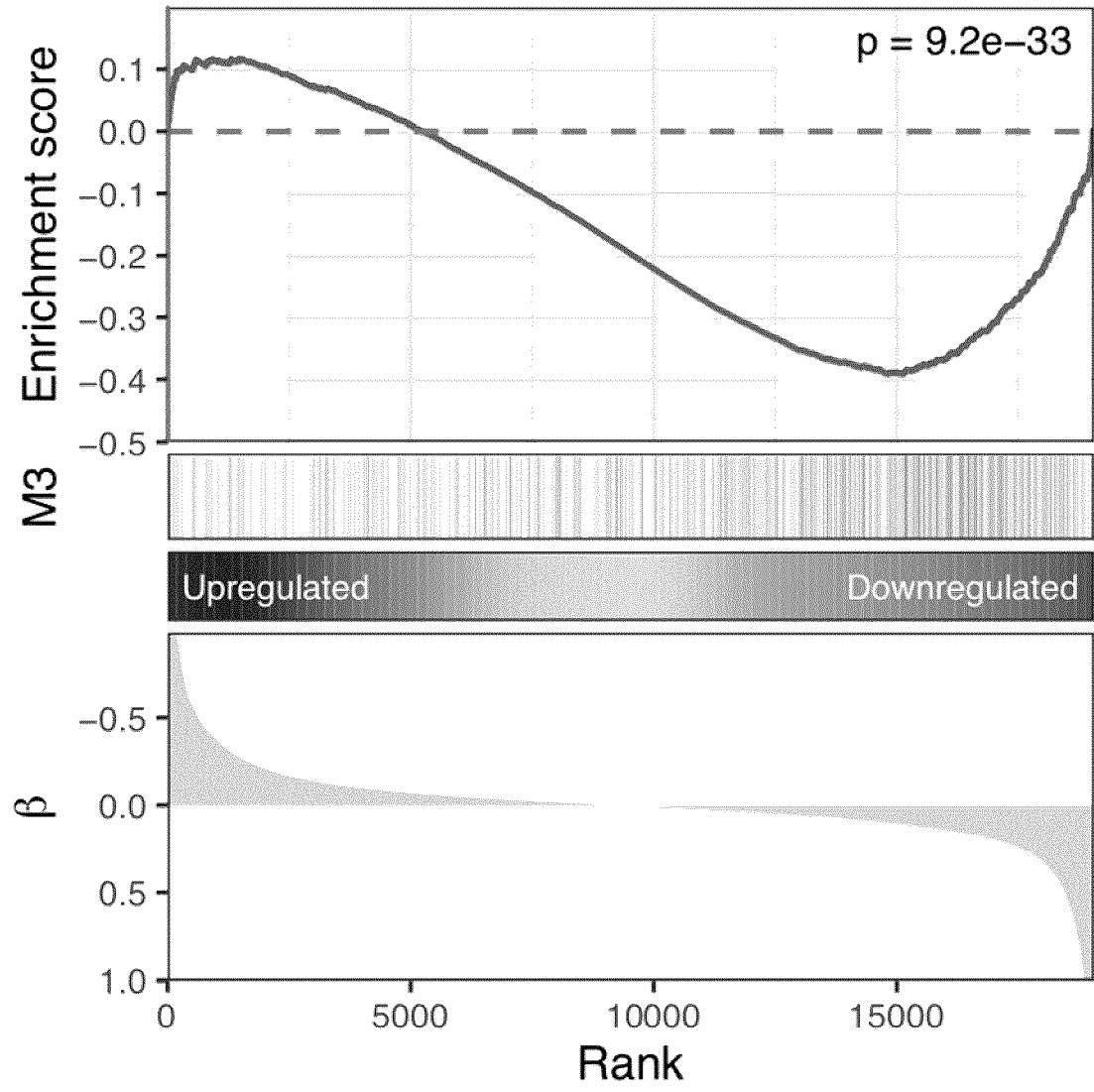
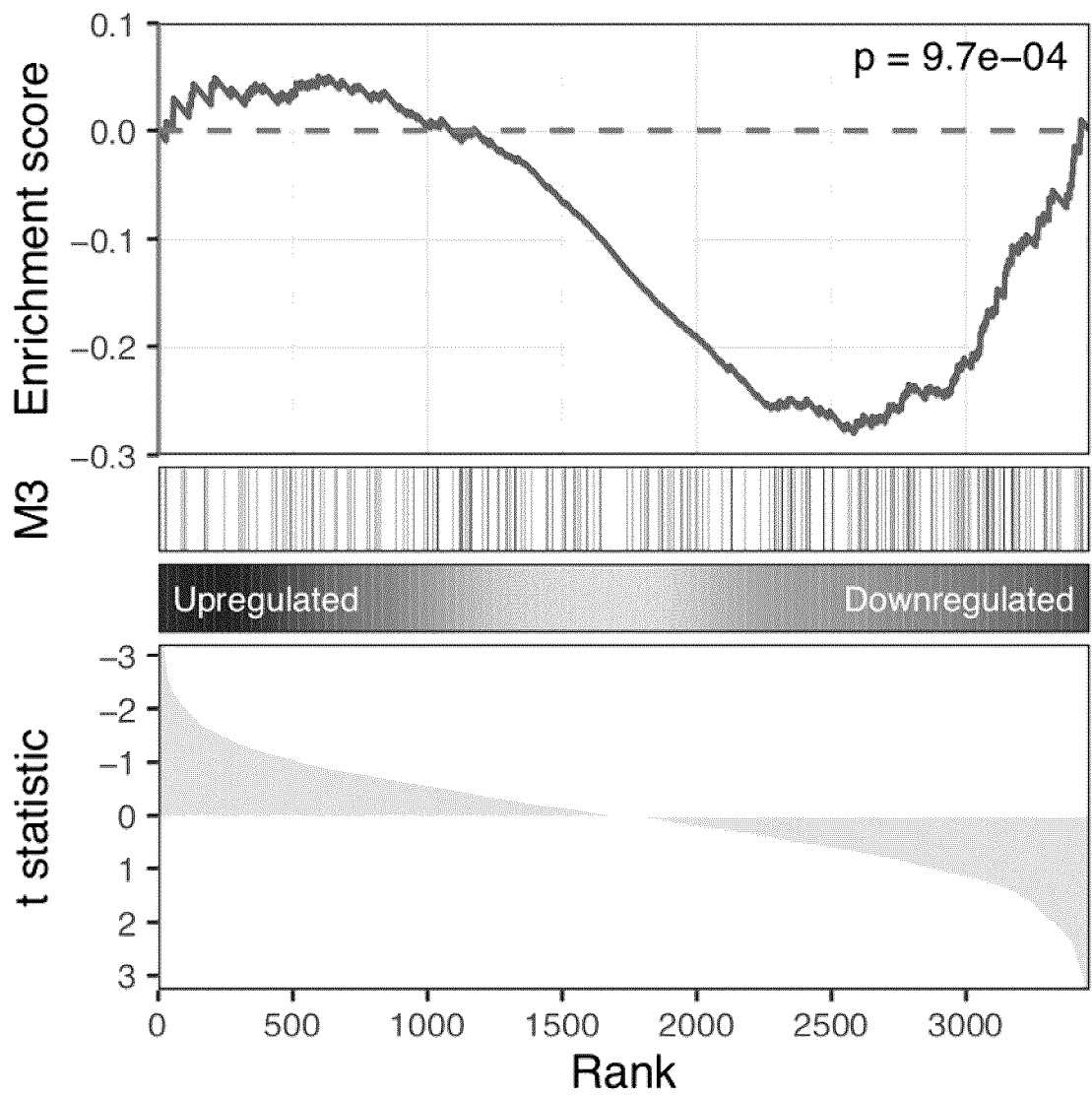


FIG. 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2019/051226

A. CLASSIFICATION OF SUBJECT MATTER
 IPC: **A61K 31/635** (2006.01), **A61P 25/00** (2006.01), **C07D 231/42** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **A61K 31/635** (2006.01), **A61P 25/00** (2006.01), **C07D 231/42** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

PubMed, STN (CAPlus, WPINDEX), CIP0 Library Discovery Tool, Questel Orbit, Scopus

Search terms: sulfonamide, acetazolamide, acetohexamide, amprenavir, apricoxib, asunaprevir, azabon, beclabuvir, bosentan, brinzolamide, bumetanide, carbutamide, celecoxib, chlorpropamide, chlorthalidone, clopamide, darunavir, dasabuvir, delavirdine, dofetilide, dorzolamide, dronedarone, ethoxzolamide, fosamprenavir, furosemide, blibenclamide, glibornuride, gliclazide, glycolopyramide, glimepiride, glipizide, gliquidone, glisoxepide, grazoprevir, hydrochlorothiazide, ibutilide, indapamide, mafenide, mefruside, methazolamide, metolazone, parecoxib, paritaprevir, probenecid, simeprevir, sotalol, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfadimidine, sulfadoxine, sulfafurazole, sulfamethoxazole, sulfamethoxypyridazine, sulfametopyrazine, sulfametoxydiazine, sulfamoxole, sulfanitrane, sulfaphenazole, sulfasalazine, sulfisomidine, sultiame, sumatriptan, tamsulosin, terephthyl, tipranavir, tolazamide, tolbutamide, udenafil, xipamide, zonisamide, Central nervous system, trauma, brain injury, spinal cord injury

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAYRI HAMA, et al. "Effects of Sulfaphenazole after Collagenase-Induced Experimental Intracerebral Hemorrhage in Rats" <i>Biological and Pharmaceutical Bulletin</i> 35(10) 1849-1853 (2012) ISSN: 0918-6158 *Whole document*	1-3, 6, 17-19, 22
X	US 2008/0045611 A1 (RADOJICIC) 21 February 2008 (21-02-2008) *Whole document*	1, 2, 4, 6, 17, 18, 20, 22
X	CA 2 999 414 A1 (OHNO et al.) 06 April 2017 (06-04-2017) *Examples, results; pages 60-61*	1, 2, 4, 6, 17, 18, 20, 22

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search

27 September 2019 (27-09-2019)

Date of mailing of the international search report

06 November 2019 (06-11-2019)

Name and mailing address of the ISA/CA
 Canadian Intellectual Property Office
 Place du Portage I, C114 - 1st Floor, Box PCT
 50 Victoria Street
 Gatineau, Quebec K1A 0C9
 Facsimile No.: 819-953-2476

Authorized officer

May Ling Nung (819) 639-9347

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2019/051226**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group A – Claims 1-6, 17-22 are directed to use of a sulfonamide for treatment of central nervous system trauma and a method of medical treatment; and

Group B - Claims 7-16 are directed to a method of identifying a compound suitable for treatment of central nervous system trauma and use of the identified compound for treatment of central nervous system trauma.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

1-6, 17-22

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2019/051226

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/72308 A1 (GWAG et al.) 04 October 2001 (04-10-2001) *Examples 1-4; claims*	1, 2, 4-6, 17, 18, 20-22
X	WO 2008/098160 A1 (SIMARD et al.) 14 August 2008 (14-08-2008) *Abstract; paragraphs 0026, 0027; Claims 1-3, 14*	1, 2, 4-6, 17, 18, 20-22
X	WO 2017/062765 A1 (JACOBSON et al.) 13 April 2017 (13-04-2017) *page 4, lines 11-14; page 7 lines 12-15; Example 2; claims 1, 5, 13, 18, 20, 73, 111*	1, 2, 5, 6, 17, 18, 21, 22
X	US 2003/0059476 A1 (WANG) 27 March 2003 (27-03-2003) *paragraphs 0029, 0030, 0044, 0045; Examples 1-3; Claims 7, 14*	1, 2, 4-6, 17, 18, 20-22
X	US 2007/0270449 A1 (BARLOW et al.) 22 November 2007 (22-11-2007) *Abstract; paragraphs 0010, 0021, 0395-6; Example 19; Claims 15, 18, 23*	1, 2, 4-6, 17, 18, 20-22
X	WO 2005/037193 A2 (STEPHENSON et al.) 28 April 2005 (28-04-2005) *Abstract; paragraphs 0017-0021, 0156-0159; claims 1, 4, 6, 15*	1, 2, 4-6, 17, 18, 20-22
X	US 2012/0148560 A1 (DAWSON-SCULLY et al.) 14 June 2012 (14-06-2012) *paragraphs 0026, 0032, 0033; claims 10, 12, 13, 17*	1, 2, 4, 6, 17, 18, 20-22
X	US 2018/0161339 A1 (FOSSATI) 14 June 2018 (14-06-2018) *paragraphs 0014-0016, 0026, 0028, 0029, 0041, 0048, 0082, 00117-00153, 00184-202; Example 1; Claims 1, 8, 9, 14, 28*	1, 2, 5, 6, 17, 18, 21, 22
X	DAHL et al. "Pannexin: From discovery to bedside in 11+4 years?" <i>Brain Research</i> 1487 (2012) 150-159. *Abstract; page 155, Section 2.6*	1, 2, 6, 17, 18, 22
X	US 2006/276411 A1 (SIMARD et al.) 07 December 2006 (07-12-2006) *paragraphs 28, 256, 304-310, 312, 313; claims 9, 22, 29, 34, 35*	1, 2, 4-6, 17, 18, 20-22
A	BERAKI et al. "A pharmacological screening approach for discovery of neuroprotective compounds in ischemic stroke" <i>PLoS One</i> (2013), 8(7), e69233 CODEN: POLNCL; ISSN: 1932-6203 *Whole document*	1-6, 17-22
A	VOIROL et al. "Cytochrome P-450 activities in human and rat brain microsomes" <i>Brain Research</i> 855 (2000) 235-243. *Whole document*	1-6, 17-22
A	KOWALSKI et al. "Acute Ischemic Stroke After Moderate to Severe Traumatic Brain Injury Incidence and Impact on Outcome" <i>Stroke</i> 48(7), 1802-1809 *Whole document*	1-6, 17-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2019/051226

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2008045611A1	21 February 2008 (21-02-2008)	US2012271168A1 US9463276B2 US2009227851A1 US9770180B2 US2008044473A1 US2008045883A1 US2010312084A1 WO2013159072A1	25 October 2012 (25-10-2012) 11 October 2016 (11-10-2016) 10 September 2009 (10-09-2009) 26 September 2017 (26-09-2017) 21 February 2008 (21-02-2008) 21 February 2008 (21-02-2008) 09 December 2010 (09-12-2010) 24 October 2013 (24-10-2013)
CA2999414A1	06 April 2017 (06-04-2017)	CN108135888A EP3357494A1 EP3357494A4 JPWO2017057562A1 US2018271838A1 WO2017057562A1	08 June 2018 (08-06-2018) 08 August 2018 (08-08-2018) 22 May 2019 (22-05-2019) 02 August 2018 (02-08-2018) 27 September 2018 (27-09-2018) 06 April 2017 (06-04-2017)
WO0172308A1	04 October 2001 (04-10-2001)	AU4150000A AU780893B2 CA2404901A1 CN1452490A EP1284739A1 JP2003528145A KR20010092980A KR100417623B1 US6521640B1	08 October 2001 (08-10-2001) 21 April 2005 (21-04-2005) 04 October 2001 (04-10-2001) 29 October 2003 (29-10-2003) 26 February 2003 (26-02-2003) 24 September 2003 (24-09-2003) 27 October 2001 (27-10-2001) 05 February 2004 (05-02-2004) 18 February 2003 (18-02-2003)
WO2008098160A1	14 August 2008 (14-08-2008)	CA2618099A1 CA2618099C EP2114160A1 EP2114160A4 EP2114160B1 US2010092469A1 US2019111137A1	09 August 2008 (09-08-2008) 20 September 2016 (20-09-2016) 11 November 2009 (11-11-2009) 11 December 2013 (11-12-2013) 16 November 2016 (16-11-2016) 15 April 2010 (15-04-2010) 18 April 2019 (18-04-2019)
WO2017062765A1	13 April 2017 (13-04-2017)	AU2016335767A1 AU2016335767A8 BR112018006925A2 CA3001321A1 CN108348534A EA201890893A1 EP3359162A1 EP3359162A4 IL258509D0 JP2018530564A KR20180063296A MX2018004286A US2018280325A1	24 May 2018 (24-05-2018) 28 June 2018 (28-06-2018) 16 October 2018 (16-10-2018) 13 April 2017 (13-04-2017) 31 July 2018 (31-07-2018) 28 September 2018 (28-09-2018) 15 August 2018 (15-08-2018) 28 August 2019 (28-08-2019) 31 May 2018 (31-05-2018) 18 October 2018 (18-10-2018) 11 June 2018 (11-06-2018) 09 August 2018 (09-08-2018) 04 October 2018 (04-10-2018)
US2003059476A1	27 March 2003 (27-03-2003)	US6683066B2 AU2002335736A1 US2004138125A1 US2004142905A1 US2004142906A1 WO03026565A2 WO03026565A3	27 January 2004 (27-01-2004) 07 April 2003 (07-04-2003) 15 July 2004 (15-07-2004) 22 July 2004 (22-07-2004) 22 July 2004 (22-07-2004) 03 April 2003 (03-04-2003) 20 November 2003 (20-11-2003)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2019/051226

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2007270449A1	22 November 2007 (22-11-2007)	US7678808B2 AU2007249435A1 CA2651862A1 EP2026813A2 JP2009536667A MX2008014320A US2009325949A1 US2010009983A1 WO2007134077A2 WO2007134077A3	16 March 2010 (16-03-2010) 22 November 2007 (22-11-2007) 22 November 2007 (22-11-2007) 25 February 2009 (25-02-2009) 15 October 2009 (15-10-2009) 25 March 2009 (25-03-2009) 31 December 2009 (31-12-2009) 14 January 2010 (14-01-2010) 22 November 2007 (22-11-2007) 03 January 2008 (03-01-2008)
WO2005037193A2	28 April 2005 (28-04-2005)	WO2005037193A8 WO2005037193A3 BRPI0414956A CA2540623A1 EP1670417A2 JP2007511468A MXPA06003663A US2005113434A1 US2007149591A1	06 April 2006 (06-04-2006) 21 December 2006 (21-12-2006) 14 November 2006 (14-11-2006) 28 April 2005 (28-04-2005) 21 June 2006 (21-06-2006) 10 May 2007 (10-05-2007) 05 June 2006 (05-06-2006) 26 May 2005 (26-05-2005) 28 June 2007 (28-06-2007)
US2012148560A1	14 June 2012 (14-06-2012)	US2011306556A1 US2015202219A1	15 December 2011 (15-12-2011) 23 July 2015 (23-07-2015)
US2018161339A1	14 June 2018 (14-06-2018)	None	
US2006276411A1	07 December 2006 (07-12-2006)	US8980952B2 AT486606T AT487484T AU2003222020A1 AU2003222020B2 AU2005287090A1 AU2005290238A1 AU2008243265A1 AU2008243265B2 AU2011201252A1 AU2011201252B2 CA2477812A1 CA2477812C CA2580606A1 CA2583397A1 CA2583397C CA2643360A1 CA2643360C CN101043891A CN101217962A CN101217962B DE602005024597D1 DE602005024704D1 DK1529058T3 EP1529058A2 EP1529058A4 EP1529058B1 EP1799227A2 EP1799227A4 EP1799227B1 EP1802313A2 EP1802313B1	17 March 2015 (17-03-2015) 15 November 2010 (15-11-2010) 15 November 2010 (15-11-2010) 08 October 2003 (08-10-2003) 28 August 2008 (28-08-2008) 30 March 2006 (30-03-2006) 06 April 2006 (06-04-2006) 04 December 2008 (04-12-2008) 23 December 2010 (23-12-2010) 07 April 2011 (07-04-2011) 06 December 2012 (06-12-2012) 02 October 2003 (02-10-2003) 14 October 2014 (14-10-2014) 30 March 2006 (30-03-2006) 06 April 2006 (06-04-2006) 21 May 2013 (21-05-2013) 07 September 2007 (07-09-2007) 11 October 2016 (11-10-2016) 26 September 2007 (26-09-2007) 09 July 2008 (09-07-2008) 11 May 2011 (11-05-2011) 16 December 2010 (16-12-2010) 23 December 2010 (23-12-2010) 02 December 2013 (02-12-2013) 11 May 2005 (11-05-2005) 30 July 2008 (30-07-2008) 06 November 2013 (06-11-2013) 27 June 2007 (27-06-2007) 24 September 2008 (24-09-2008) 10 November 2010 (10-11-2010) 04 July 2007 (04-07-2007) 03 November 2010 (03-11-2010)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2019/051226

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		EP1993562A4	18 July 2012 (18-07-2012)
		EP2359832A2	24 August 2011 (24-08-2011)
		EP2359832A3	23 November 2011 (23-11-2011)
		EP2382977A1	02 November 2011 (02-11-2011)
		EP2382977B1	06 August 2014 (06-08-2014)
		EP2438913A1	11 April 2012 (11-04-2012)
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		IL181740D0	13 April 2008 (13-04-2008)
		IL181826D0	13 April 2008 (13-04-2008)
		JP2005534285A	17 November 2005 (17-11-2005)
		JP4485806B2	23 June 2010 (23-06-2010)
		JP2008513478A	01 May 2008 (01-05-2008)
		JP5085326B2	28 November 2012 (28-11-2012)
		JP2008513447A	01 May 2008 (01-05-2008)
		JP5307397B2	02 October 2013 (02-10-2013)
		JP2009527579A	30 July 2009 (30-07-2009)
		JP2012017346A	26 January 2012 (26-01-2012)
		JP2012056966A	22 March 2012 (22-03-2012)
		US2005181980A1	18 August 2005 (18-08-2005)
		US7285574B2	23 October 2007 (23-10-2007)
		US2006100183A1	11 May 2006 (11-05-2006)
		US7872048B2	18 January 2011 (18-01-2011)
		US2008139659A1	12 June 2008 (12-06-2008)
		US8318810B2	27 November 2012 (27-11-2012)
		US2011092542A1	21 April 2011 (21-04-2011)
		US8569377B2	29 October 2013 (29-10-2013)
		US2014235564A1	21 August 2014 (21-08-2014)
		US9107932B2	18 August 2015 (18-08-2015)
		US2003215889A1	20 November 2003 (20-11-2003)
		US2009130083A1	21 May 2009 (21-05-2009)
		US2009137680A1	28 May 2009 (28-05-2009)
		US2012237449A1	20 September 2012 (20-09-2012)
		US2015272964A1	01 October 2015 (01-10-2015)
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