# PainNetworks: A web-based resource for the visualisation of pain related genes in the context of their network associations

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## *Abstract*

We now know of hundreds of genes that have some association with pain. Several genes have been shown to alter pain sensitivity in humans, and can be found in the OMIM database. The Pain Gene Database gives details of genes that have been shown to alter pain-related behaviour in transgenic mouse models (usually gene knockout). Genes identified in this way are often studied in isolation, or alongside a handful of other genes. Techniques from systems biology, and methods for identifying protein interactions and gene associations using data derived from functional genomics studies allow us to study these genes in the context of the biological systems and pathways on which they operate. Predicted gene associations, generated by various bioinformatics tools, can be used to extend these associations and enrich the information available on protein networks.

Here we describe a resource, available at www.painnetworks.org,that allows the user to visualise pain genes in the context of an interaction network. The user can also enrich the networks using data from a number of pain-focused gene expression studies to highlight genes that change in expression in a given experiment and genes showing correlated patterns of expression in a number of different experiments. The website currently contains several pain-related datasets and the user is able to input their own experiment to view alongside these datasets (without the need to send any of their data over the web). We also invite users to submit their own data to the website. We expect this resource to grow over time and become a valuable asset to the pain community.

## *Introduction*

We have discovered much about pain and nociception using in vivo animal studies. Knock out mice, pain models and related approaches have led to advances in our understanding of the genes involved in pain [1,20,27,34]. However, many of the drugs designed to target single proteins have shown a lack of efficacy when trialled in humans and led to unforeseen side-effects [46]. This may be due to the polygenic nature of pain. It has a highly heterogeneous physiologic and molecular basis - at least ten independent (albeit overlapping) mechanisms have been proposed for the initiation and sustenance of pain [15,46]. Recent work suggests there may be many more pain-associated genes waiting to be discovered [34]. Pain researchers often consider a single aspect of pain in isolation, perhaps a given physiological process or group of genes. However it seems clear that systems biology, defined as “the strategy of pursuing integration of complex data about the interactions in biological systems from diverse experimental sources using interdisciplinary tools and personnel” [7], will become an important and necessary paradigm for the future study of pain, and lead to a greater success in the development of analgesics [4].

Microarrays represent one way in which pain has been studied at a system-wide level [13,24,26,32]. However most microarrays give a snap-shot of pain, specific to a given tissue type, for a given animal or human model of pain, at a given point in time, often long after the pain generating insult has occurred [4]. A further way to study pain is at the systems level, by combining the data available on the interactions and associations of pain genes to produce networks. Such approaches have been successfully applied in many research areas outside pain, including cancer [11,36,42], diabetes [30] and asthma [21]. Closer to the fields of pain and neuroscience, module-based methods have been used to study neurological disorders including Parkinson’s [35] and Alzheimer’s [25,39], and systemic inflammation. Several websites and resources exist that allow a researcher to build such networks, in order to examine their gene(s) of interest in the context of their protein associations. These include both experimentally detected interactions and inferred/predicted associations [28,41,45].

Here we present a publically available resource, PainNetworks.org, aimed at pain researchers, that allows the user to build pain specific networks, integrating microarray data from a wide range of pain related experiments, profiling expression changes in animal models of pain and knock-out mice. The user can compare their experimental data to that included in the PainNetworks.org site, client-side, without uploading anything to the web. The user can also cluster their constructed networks using node and edge based clustering methods, request various functional annotations, such as GO categories and known drug targets, and filter the networks to only include genes from a specific tissue-type or anatomical structure of interest, based on tissue-specific expression data. In addition to customising the content of their network, the user can move the protein components around on the screen interactively, allowing them to focus on whatever part of the network they wish to examine further.

In this short paper we will describe the website and the underlying methodology used to provide its features. We will also present some example usages of the site. A more detailed reference manual is available from the site itself, showing the different features of the sites and explaining how they can be used. Video tutorials and example queries are also available from the site.

## *Methods*

### Pain related genes

The user can input a set of query genes in order to produce a network. However the website also contains lists of pain related genes sourced from public sources, namely 1) the Online Mendelian Inheritance in Man (OMIM) database, which is an online database of diseases and related genes (containing traditional "Mendelian" diseases and more complex polygenic diseases) [3],and 2) the Pain Genes Database (PGD) maintained by Jeffery Mogil's Pain Genetic Lab, which contains genes that, according to a published study, lead to a change in pain-related behaviour when knocked out in mouse [27]. PainNetworks.org will be updated regularly in order to capture any additional genes placed in either the PGD, OMIM or other published pain based resources. The user can use genes from these resources to annotate their network, or to identify clusters in the network with an over-representation of pain-associated genes, as described below. These pain-related genes can come from two different sources.

### Interaction/association data

Protein interactions were taken from eight public databases (IntAct, MINT, MIPS, BIOGRID, DIP, HPRD, and Reactome [5,12,16,18,23,37,40] and combined to produce a protein interaction network. Proteins were mapped to their genes, allowing the data from different databases to be combined using a common identifier. This allowed us to resolve ambiguous mapping between databases. Current technology identifying protein interactions is such that the specific splice variant of the gene involved in the interaction is only known in a small minority of cases and so combining at the gene level does not lead to much loss of information. Direct protein-protein interactions (such as interactions curated from the literature or found using yeast-2-hybrid screens) are displayed in the network as solid lines. Indirect associations (such as associations inferred through co-complex membership) are displayed as broken lines, representing the reduced confidence in these interactions.

The focus of PainNetworks.org is on experimentally defined interactions. However we also provide the user with the ability to add prediction interactions from the String resource [41], v 9.0. As with many features of the website this option is not enabled by default, but can be selected by the user and may be valuable for poorly annotated, less well studied genes for which few interactions are known.

## Microarray data and analysis

The currently available datasets in the website consist of microarray data taken from 4 animal pain models,, collected by researchers in the London Pain Consortium and other pain researchers based in London. Several of these are published [13,14]; Details on experimental design have either been published or are provided on the website and are summarized in Table 1. Further details of all currently available experiments can be viewed by clicking on the experiments tab. This allows lists of genes and fold changes to be downloaded for each experiment, in a variety of formats, or browsed online. More datasets will be added in time and we invite members of the pain community to upload datasets to the site. Users can also view their own expression datasets through the site locally (client side), without having to send any data via the web.

All microarray datasets were produced using Affymetrix technology. Microarray data was pre-processed using the RMA package [10,22]. Differential gene expression was calculated using limma [38], which produced a p-value for each gene probed by the array. False discovery rate (FDR) was estimated from these p-values using the Benjamini-Hochberg method [8]. Fold change in gene expression were computed between case samples (i.e. animals subjected to the model, or knock-out animals) and control samples (i.e. the corresponding control for the model, or wild type animals for the knock-out models). An FDR of 0.1 was accepted when deciding if a gene is differentially expressed between case and control samples.

## Filtering to obtain context specific networks

Networks can be filtered using various methods to make the networks more context-specific. For example a list of Gene ontology [6] (GO) IDs can be entered, and only genes annotated with one or more of the selected terms would be retained in the network. In this way networks can be filtered to only display genes involved in specific biological processes (e.g. GO:0006954, inflammatory response), molecular functions (e.g. GO:0005244, voltage-gated ion channel activity) or expressed in given cellular components (e.g. GO:0045202, synapse).

Additionally the user can filter to select genes differentially expressed in one or more of the microarray experiments. Another option is to filter the network so that it displays interactions where both genes show significant differential expression (in the same direction) in a given number of experiments. Such filtering can greatly reduce the size of an interaction network, making it easier to examine and interpret, and make the network more pain relevant. It is also provides a way to combine and visualise different microarray datasets. The filtering can also be applied to networks that include predicted interactions.

Networks can also be filtered using tissue specific expression data, in order to produce networks that only contain genes expressed in a given tissue/anatomical structure. This is taken from the Gene Expression Database (GXD)[19]. This database combines large compendia of gene expression data in several anatomical systems and structures in mice produced using

various techniques including  immunohistochemistry, in situ hybridisation and RT-qPCR [19].. The user is able to filter by genes expressed in several different anatomical structures, such as the dorsal root ganglion, the dorsal horn, skin, immune cells such as macrophages, and various nerves. The user can also filter using higher-level structures, for example the nervous or immune system.

The website has been designed to allow the user to use the GXD data as a search option (i.e. the user can search specifically using genes expressed in a given tissue) or to filter resultant networks, in which case all genes not expressed in the selected tissue would be removed from the network, along with their interactions.

## Clustering the network

Since there is a large amount of experimental data available for many genes (some of which may be noise), it is very easy to generate large networks (i.e. greater than 100 nodes), which are difficult to interpret. Therefore PainNetworks.org allows the user to cluster the network. The advantages of clustering are twofold: to help visualisation, and to find modules enriched in specific functions. We use established module detection algorithms, the aim of which is to find groups in the network with a high number of interactions between group members, but a small number of interaction between members of the group and the other genes in the network outside the group.

Two methods are implemented to look for clusters in the network, Louvain clustering [9] and Links clustering [2]. Louvain looks for distinct clusters of genes in the network, not allowing a given gene to belong to more than one network. Links clustering groups the edges of the network, i.e. the interactions. This second approach has the potential advantage that it allows communities to be found with overlapping nodes, the same gene can belong to different clusters in the network. This fits in well with the functional pleiotropy of many genes.

Once clusters have been built they can be ranked according to their size or the number of pain related genes they contain. Enriched GO categories for each cluster are also displayed.

## Transferring annotation between species

The known pain genes deposited in the Pain Genes Database are associated with a change in pain-related behaviour when knocked out in mouse [27]. The protein protein interaction (PPI) and OMIM data is largely from humans, though the PPI data includes data from other species. The pain related microarray data is largely from rat or mouse and the tissue-specific expression data is from mouse. To integrate the data, annotations must be transferred to one species. PainNetworks.org currently allows the data to be transferred to mouse, rat or human. Thus there are three different possible views of the site, human-centric, mouse-centric or rat-centric.

The Ensembl Compara method, for identifying orthologues, is used to map genes between species [44].

In order to transfer PPIs between species, a method similar to the interlogs procedure [33] is used, which we name Homologous Inheritance of Protein Protein interactions by Orthology-Conservative (HIPPO-C): Given an interaction between a pair of genes in one species, if both of these genes have unambiguous orthologues in another species, this interaction can be inherited between species. Inherited interactions will be visualised as a green edge in the network, as opposed to black for interactions not inherited from other species. For example, when looking at the mouse interaction network (i.e. using the mouse centred view), inherited interactions found using human-based methodology would be coloured green; interactions discovered using mouse-based methodology would be coloured black.

We also include a further method for protein interaction inheritance, named HIPPO-DB (Homologous Inheritance of Protein Protein interactions by Orthology-Domain Based). This method uses the CATH superfamily classifications [17,29] to identify the likely subset of domain interactions underpinning the protein interactions. Interactions are transferred when their domain interactions are consistent with this subset (paper under revision).

Predicted gene associations taken from the STRING database are available for the human centric view, and are not transferred between species. Only high confidence predictions (i.e. with a STRING score > 400) are included.

### Implementation

The website was implemented using Python and CSS. Cytoscapeweb, a Flash-based plugin [31], available from http://cytoscapeweb.cytoscape.org/ was used to visualise the networks.

## *Results and Discussion*

Here we show the numbers of interactors for the genes within the network, and how that corresponds to the proportion of interactors that are pain-related. followed by some example usage scenarios for the site. However, the best way to get to know the site is to use it. We have tried to make the site as intuitive as possible, however there is a large amount of functionality and a number of ways to view the networks and export the data. We will not document all of the different features of the site here, since there is a reference manual available from the site at www.PainNetworks.org/RefMan.pdf. This manual describes how to use all of the features of the site, illustrated with screenshots. There are also video tutorials, available at http://www.youtube.com/channel/UCfl06Zr51Sy3BgN9eca_w0A/videos?view=0, which present potential usage scenarios. It is also possible to search the site using example queries presented on the site itself, easily accessible by

clicking on the “Click for simple tutorial” link, located at the top right of the homepage.

A non-exhaustive summary of potential uses for the site is also presented in Table 2. We describe three such potential uses in further detail below:

## Total number of interactions for each gene in the network, compared to the number of pain genes they interact with

Figure 1 shows, for each gene in the database for which an Ensembl ID is known, and for which at least one interaction is known, the number of interactions each gene has (network degree), and the number of its interactions that are pain related. There is a general increase in the number of pain related interactors as the total number of interactions increases. However we see a division, with many genes showing much increase in pain related interactors with increasing degree, and others showing a much faster increase. The red genes represent genes with pain-related annotation. We see that many of these genes are included in the group of genes that show a fast increase in pain related genes interactions with increasing network degree. This figure is produced at the human level, that is, annotations and interactions have been transferred to humans, using the methods described above.

## Finding more about genes of interest and their interactors

In Figure 2 (Fig2.pdf) we show some basic analyses of the four different human opiod receptors, the δ opiod receptor, κ opioid receptor, μ opioid receptor and nociceptin receptor. The top of Figure 1 shows a basic search of the website using the genes that encode these receptors, OPRD1, OPRK1 OPRM1 and ORL1, in the human-centric view. This results in a very large network, too large to interpret visually. Therefore only interactions with the query genes are shown in the network; interactions between the interactors of the query genes are not shown. Three further analyses to refine the network are shown in the bottom half of the figure. On the left one of the clusters found using the Louvain clustering is shown. The Louvain method partitions the genes into separate, non-overlapping clusters. We see that two of the opioid receptors are placed into a cluster with guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O (GNAO) and a number of other genes from the pain genes database (Figure 1a). Druggable targets are then highlighted (Figure 1b). The middle network (Figure 1c) shows the results of clustering using the links based method, we see a highly connected subnetwork between a number of genes including several pain genes and some chemokines. The right-most network (Figure 1d) network shows the results of filtering the network to only show direct physical interactors (not co-complex membership) of the opioid receptors and the interactions between them.

*Fig2 Caption: Looking for druggable proteins associated with opioid receptors. Building the network using all four opioid receptors results in a large network. Different ways of reducing the network to make it more interpretable are presented and explained further in the text.*

Similarly, Figure 3 (Fig3.pdf) shows how we can use PainNetworks.org to find out more about another gene, angiotensin II receptor, type 2 (AGTR2). Recent studies have shown in a phase II clinical trial that an inhibitor of this receptor (EMA 401) is effective in reducing pain experienced by suffers of postherpetic neuralgia [43]. In figure 2A we show that a large number of the interactors of AGTR2 are also pain-related. 73 of its 278 interactors are pain related or 27%. This proportion of interactors that are pain related is higher than 96% of the genes in the network. Figure 2B shows the network formed from all direct physical interactions involving AGTR2, AGTR1 (angiotensin II receptor, type 1) and AGTRAP (angiotensin II receptor-associated protein), onto which differentially expressed genes from the SNT microarray experiment have been painted. We notice that the interactors of AGTR2 are all differentially expressed, suggesting a potential role of this pathway in neuropathic pain.

*Fig3 Caption: Finding out more about angiotensin II type 2 receptors. A: Looking at the direct and indirect interactors. Note that the shaded circles represent pain-associated genes. B: The direct physical interactors of AGTR1, AGRT2 and AGTRAP. Using the results of a microarray experiment of a model of neuropathic pain, we see many members of this pathway are significantly differentially expressed.*

## Using the results of a microarray experiment to find out more about a given gene

Cyclic AMP-dependent transcription factor (ATF3) has been shown to change in expression in a number of microarray models of neuropathic pain [26]. Figure 4 (Fig4.pdf) shows ATF3 alongside its network associations/interactions (top). Although ATF3 shows differential expression in a number of different neuropathic pain models, relatively few of its interactions are known to be associated with pain. We only see 1 pain-model gene, NFKB1, interacting with ATF3. However, if we click on the “Fold Change” link for the spinal nerve transection, transected L5 nerve vs. sham experiment, we see that many of ATF3's network interaction partners are differentially expressed in this experiment. As we would expect, many of these differentially expressed interaction partners are also transcription factors. This suggests that spinal nerve transection may be leading to changes in gene expression through the actions of a network of transcription factors during the induction phase of neuropathic pain.

*Fig4 Caption: ATF3 interactors that change in expression in a nerve injury model of pain. Top: the network returned by querying PainNetworks.org with ATF3. Pain-model genes, which in this case are the PGD genes, are highlighted as grey in the network. Bottom: the genes that change under the SNT L5 vs. sham dataset are highlighted in the network by the addition of red or blue rings, for increased or decreased expression (respectively) in L5 compared to sham*

To conclude, we have developed a resource for the pain community, allowing them to build networks based on their gene(s) of interest, and combine protein

interaction, gene association and expression data in order to examine pain at the systems level. We expect this resource to expand over time and to become a valuable asset to the pain community.

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## *References*

[1] Abrahamsen B, Zhao J, Asante C, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar M, Dickenson A, Wood J. The cell and molecular basis of mechanical, cold, and inflammatory pain. Science (New York, N.Y.) 2008;321:702–705.

[2] Ahn Y-Y, Bagrow J, Lehmann S. Link communities reveal multiscale complexity in networks. Nature 2010;466:761–764.

[3] Amberger J, Bocchini C, Scott A, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic acids research 2009;37:D793–796.

[4] Antunes-Martins A, Perkins JR, Lees J, Hildebrandt T, Orengo C, Bennett DLH. Systems biology approaches to finding novel pain mediators. Wiley Interdiscip Rev Syst Biol Med 2012.

[5] Aranda B, Achuthan P, Alam-Faruque Y, Armean I, Bridge A, Derow C, Feuermann M, Ghanbarian A, Kerrien S, Khadake J, Kerssemakers J, Leroy C, Menden M, Michaut M, Montecchi-Palazzi L, Neuhauser S, Orchard S, Perreau V, Roechert B, Van Eijk K, Hermjakob H. The IntAct molecular interaction database in 2010. Nucleic Acids Research 2009;38:D525–D531.

[6] Ashburner M, Ball C, Blake J, Botstein D, Butler H, Cherry J, Davis A, Dolinski K, Dwight S, Eppig J, Harris M, Hill D, Issel-Tarver L, Kasarskis A, Lewis S, Matese J, Richardson J, Ringwald M, Rubin G, Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nature genetics 2000;25:25–29.

[7] Baitaluk M. System biology of gene regulation. Biomedical Informatics 2009;569:55–87.

[8] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 1995:289–300.

[9] Blondel VD, Guillaume J-L, Lambiotte R, Lefebvre E. Fast unfolding of communities in large networks. Journal of Statistical Mechanics: Theory and Experiment 2008;2008:P10008.

[10] Bolstad B, Irizarry R, Åstrand M, Speed T. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–193.

[11] Bonifaci N, Berenguer A, Díez J, Reina O, Medina I, Dopazo J, Moreno V, Pujana MA. Biological processes, properties and molecular wiring diagrams of candidate low-penetrance breast cancer susceptibility genes. BMC medical genomics 2008;1. doi:10.1186/1755-8794-1-62.

[12] Ceol A, Chatr Aryamontri A, Licata L, Peluso D, Briganti L, Perfetto L, Castagnoli L, Cesareni G. MINT, the molecular interaction database: 2009 update. Nucleic acids research 2010;38:D532–D539.

[13] Costigan M, Befort K, Karchewski L, Griffin R, D'Urso D, Allchorne A, Sitarski J, Mannion J, Pratt R, Woolf C. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. BMC neuroscience 2002;3. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC139981/.

[14] Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert TA, Barrett L, Brenner GJ, Vardeh D, Woolf CJ, Fitzgerald M. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J. Neurosci. 2009;29:14415–14422.

[15] Costigan M, Scholz J, Woolf C. Neuropathic pain: a maladaptive response of the nervous system to damage. Annual review of neuroscience 2009;32:1–32.

[16] Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L, Caudy M, Garapati P, Gopinath G, Jassal B, Jupe S, Kalatskaya I, Mahajan S, May B, Ndegwa N, Schmidt E, Shamovsky V, Yung C, Birney E, Hermjakob H, D'Eustachio P, Stein L. Reactome: a database of reactions, pathways and biological processes. Nucleic acids research 2011;39:D691–D697.

[17] Cuff AL, Sillitoe I, Lewis T, Clegg AB, Rentzsch R, Furnham N, Pellegrini-Calace M, Jones D, Thornton J, Orengo CA. Extending CATH: increasing coverage of the protein structure universe and linking structure with function. Nucleic Acids Res. 2011;39:D420–426.

[18] Deane C, Salwiński Ł, Xenarios I, Eisenberg D. Protein Interactions. Molecular & Cellular Proteomics 2002;1:349–356.

[19] Finger J, Smith C, Hayamizu T, McCright I, Eppig J, Kadin J, Richardson J, Ringwald M. The mouse Gene Expression Database (GXD): 2011 update. Nucleic acids research 2011;39:D835–D841.

[20] Foulkes T, Wood J. Pain genes. PLoS genetics 2008;4. doi:10.1371/journal.pgen.1000086.

[21] Hwang S, Son S-W, Kim SC, Kim YJ, Jeong H, Lee D. A protein interaction network associated with asthma. Journal of theoretical biology 2008;252:722–731.

[22] Irizarry R, Bolstad B, Collin F, Cope L, Hobbs B, Speed T. Summaries of Affymetrix GeneChip probe level data. Nucleic acids research 2003;31. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC150247/.

[23] Keshava Prasad T, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan D, Sebastian A, Rani S, Ray S, Harrys Kishore C, Kanth S, Ahmed M, Kashyap M, Mohmood R, Ramachandra Y, Krishna V, Rahiman A, Mohan S, Ranganathan P, Ramabadran S, Chaerkady R, Pandey A. Human Protein Reference Database--2009 update. Nucleic acids research 2009;37:D767–D772.

[24] Ko J, Na DS, Lee YH, Shin SY, Kim JH, Hwang BG, Min B-I, Park DS. cDNA microarray analysis of the differential gene expression in the neuropathic pain and electroacupuncture treatment models. Journal of biochemistry and molecular biology 2002;35:420–427.

[25] Krauthammer M, Kaufmann C, Gilliam C, Rzhetsky A. Molecular triangulation: bridging linkage and molecular-network information for identifying candidate genes in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 2004;101:15148–15153.

[26] LaCroix-Fralish M, Austin J-S, Zheng F, Levitin D, Mogil J. Patterns of pain: meta-analysis of microarray studies of pain. Pain 2011;152:1888–1898.

[27] Lacroix-Fralish M, Ledoux J, Mogil J. The Pain Genes Database: An interactive web browser of pain-related transgenic knockout studies. Pain 2007;131:3.e1–3.e4.

[28] Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008;9:559.

[29] Lees J, Yeats C, Perkins J, Sillitoe I, Rentzsch R, Dessailly BH, Orengo C. Gene3D: a domain-based resource for comparative genomics,

functional annotation and protein network analysis. Nucleic Acids Res. 2012;40:D465–471.

[30] Liu M, Liberzon A, Kong SW, Lai W, Park P, Kohane I, Kasif S. Network-based analysis of affected biological processes in type 2 diabetes models. PLoS genetics 2007;3:e96.

[31] Lopes C, Franz M, Kazi F, Donaldson S, Morris Q, Bader G. Cytoscape Web: an interactive web-based network browser. Bioinformatics (Oxford, England) 2010;26:2347–2348.

[32] Maratou K, Wallace V, Hasnie F, Okuse K, Hosseini R, Jina N, Blackbeard J, Pheby T, Orengo C, Dickenson A, McMahon S, Rice A. Comparison of dorsal root ganglion gene expression in rat models of traumatic and HIV-associated neuropathic pain. European journal of pain (London, England) 2009;13:387–398.

[33] Matthews L, Vaglio P, Reboul J, Ge H, Davis B, Garrels J, Vincent S, Vidal M. Identification of potential interaction networks using sequence-based searches for conserved protein-protein interactions or “interologs”. Genome research 2001;11:2120–2126.

[34] Mogil J. Pain genetics: past, present and future. Trends in genetics : TIG 2012;28:258–266.

[35] Moran L, Graeber M. Towards a pathway definition of Parkinson’s disease: a complex disorder with links to cancer, diabetes and inflammation. Neurogenetics 2008;9:1–13.

[36] Nibbe R, Markowitz S, Myeroff L, Ewing R, Chance M. Discovery and scoring of protein interaction subnetworks discriminative of late stage human colon cancer. Molecular & cellular proteomics : MCP 2009;8:827–845.

[37] Pagel P, Kovac S, Oesterheld M, Brauner B, Dunger-Kaltenbach I, Frishman G, Montrone C, Mark P, Stümpflen V, Mewes H-W, Ruepp A, Frishman D. The MIPS mammalian protein-protein interaction database. Bioinformatics (Oxford, England) 2005;21:832–834.

[38] Smyth G. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Statistical applications in genetics and molecular biology 2004;3. doi:10.2202/1544-6115.1027.

[39] Soler-López M, Zanzoni A, Lluís R, Stelzl U, Aloy P. Interactome mapping suggests new mechanistic details underlying Alzheimer’s disease. Genome Research 2011;21:364–376.

[40] Stark C, Breitkreutz B-J, Chatr-Aryamontri A, Boucher L, Oughtred R, Livstone M, Nixon J, Van Auken K, Wang X, Shi X, Reguly T, Rust J, Winter A, Dolinski K, Tyers M. The BioGRID Interaction Database: 2011 update. Nucleic acids research 2011;39:D698–D704.

[41] Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen L, Von Mering C. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic acids research 2011;39:D561–D568.

[42] Taylor I, Linding R, Warde-Farley D, Liu Y, Pesquita C, Faria D, Bull S, Pawson T, Morris Q, Wrana J. Dynamic modularity in protein interaction networks predicts breast cancer outcome. Nature Biotechnology 2009;27:199–204.

[43] Tom D. McCarthy, Desem Nuket, Geoff Kitson, Andrew S.C. Rice, Bob H. Dwokin, Chas Bountra, Anand Praveen, Raff Milton. CLINICAL DEVELOPMENT OF EMA401; AN ANGIOTENSIN II TYPE 2 (AT2) RECEPTOR ANTAGONIST AS A POTENTIAL NEW TREATMENT FOR NEUROPATHIC PAIN. IASP 14th World Congress, Milan 2012.

[44] Vilella A, Severin J, Ureta-Vidal A, Heng L, Durbin R, Birney E. EnsemblCompara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. Genome Research 2009;19:327–335.

[45] Warde-Farley D, Donaldson S, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader G, Morris Q. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic acids research 2010;38:W214–W220.

[46] Woolf C. Overcoming obstacles to developing new analgesics. Nature medicine 2010;16:1241–1247.