Research Square

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The gene regulatory network in different brain regions of neuropathic pain mouse models

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Abstract

Neuropathic pain is the direct result caused by lesions or somatosensory nervous system diseases that are associated with emotional regulation. The incidence of neuropathic pain in the general population is 7-10% and the mechanisms of neuropathic pain are largely unknown. It is often related to structural and functional abnormalities in multiple brain regions. The forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and periaqueductal gray (PAG) have been shown to correspond with the regulation of neuropathic pain. To investigate the molecular mechanism of neuropathic pain across different brain regions, we identified the differentially expressed genes between the spared nerve injury model (SNI) mice of neuropathic pain and the control Sham mice in NAc, mPFC and PAG and mapped these genes onto comprehensive functional association network. With Random Walk with Restart (RWR) analysis, we identified more novel neuropathic pain genes in NAc, mPFC and PAG, such as Asic3, Cd200r1 and MT2, beside well known Capn11 and CYP2E1. What's more, we discovered their interactions or cross talks. Our results provided novel insights of neuropathic pain and provided therapeutic targets for treating neuropathic pain.

Background

Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as the direct result caused by lesions or somatosensory nervous system diseases that are associated with emotional regulation [1,2]. The incidence of neuropathic pain in the general population is 7-10% [3] and the mechanisms of neuropathic pain are heterogeneous [4]. Many patients with neuropathic pain are also experienced depression and anxiety disorders and reduced quality of life [5,6].

Currently, recommended treatments for neuropathic pain is pharmacological, such as the use of antidepressants, anticonvulsants and topical anesthetics [7,8]. In some cases, however, medical therapy alone cannot fully control chronic pain. Some non-pharmacological approaches, including psychological approaches, physical therapy, interventional therapy and surgical procedures have been shown to be effective for neuropathic pain [8]. In addition, the distinction between nociceptive and neuropathic pain is also important because different treatment methods are usually required for different types of pain.

Chronic pain is often related to structural and functional abnormalities in the brain [9,10]. Previous studies have shown that an increase in activity of the forebrain neurons results in enhanced inflammatory and neuropathic pain [11,12]. The forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and paraventricular nucleus (PVN) have been shown to correspond with the regulation of neuropathic pain [13,14]. Nucleus accumbens (NAc) is known to be related to emotional dysfunctions following neuropathic pain regulation as a key component of the brain reward system [15-17]. Experiments made by Goffer et al. demonstrated that chronic pain induced depressive behaviors in rats and selectively increases the level of AMPA-type glutamate receptors in the NAc, suggesting a crucial role for NAc in the regulation of neuropathic pain-induced depression [18].

It has been reported that neuropathic pain leads to morphological and functional changes in the mPFC and its important role in the regulation of emotional processes and chronic pain has also been identified [19,20]. Animal and human imaging studies have proved that synaptic changes in the PFC occur in both chronic pain and acute models [21,22]. The key output target for the PFC is the nucleus accumbens (NAc). Functional connections between NAc and mPFC were also reported to predict the prognosis of chronic pain after medical treatment [23,24]. Lee et al. suggested that the activation of mPFC-NAc projections could regulate the affective symptoms of neuropathic pain [22].

Patients with chronic pain also exhibit brain abnormalities in descending modulation of pain [25,26], especially in the periaqueductal gray (PAG), which may be associated with dysfunctions of pain regulation [27-29]. Neuropathic pain activates neurons in the periaqueductal gray (PAG), the neurons projecting to the rostral ventromedial medulla (RVM) and then projected to the spinal cord to inhibit or facilitate the pain [30].

Since the brain region corresponding to chronic pain is between the ventromedial PFC and PAG in humans [31], and mPFC-PAG in rodents [32], we would like to identify the genes that repose to neuropathic pain and investigate the molecular mechanisms of neuropathic pain. The differentially expressed genes in the nucleus accumbens (NAc), the medial prefrontal cortex (mPFC), and the periaqueductal grey (PAG) of the spared nerve injury model (SNI) were mapped onto gene regulatory network. Novel neuropathic pain gene in different brain regions were identified using Random Walk with Restart (RWR) algorithm and their interactions or cross talks were analyzed.

Methods

The differentially expressed genes between SNI and Sham mice in NAc, mPFC and PAG

Descalzi et al. [33] RNA-sequenced the NAc, mPFC and PAG gene expression profiles in SNI mice and Sham mice. SNI mice was the mouse model for neuropathic pain and Sham mice was used as control. There were six Sham NAc, six Sham PAG, six Sham PFC, six SNI NAc, six SNI PAG and six SNI PFC samples. Their RPKM (Reads Per Kilobase per Million mapped reads) gene expression level can be found in GEO (Gene Expression Omnibus) under accession number of GSE91396. A strict threshold of differentially expressed fold change greater than 1.5 and p value smaller than 0.05 was applied to get the differentially expressed genes between SNI mice and Sham mice in NAc, mPFC and PAG. The p value was calculated using function voom from R package of limma. The differentially expressed genes between SNI mice and Sham mice in NAc, mPFC and PAG were mapped onto STRING network [34] for further analysis. STRING is a comprehensive protein functional association network.

The network expansion of NAc, mPFC and PAG neuropathic pain genes based on RWR analysis

As we mentioned before, we would like to investigate the interactions or cross-talks among different brain regions that were responsive to neuropathic pain. The differentially expressed genes between SNI mice

and Sham mice in NAc, mPFC and PAG were a good start for such network analysis. Therefore, we mapped these three gene lists onto the comprehensive functional association network of STRING [34], a widely used network for bioinformatics studies [35-38]. Only the high confidence interactions of STRING were included, in other words, the confidence score of the interaction must be greater than 0.900.

To explore the cross talk between brain regions, we applied Random Walk with Restart (RWR) algorithm [35,39-42]. To illustrate how RWR can reveal the cross talk, let us denote the STRING network as a graph comprised of a set of genes and a set of interactions .

The whole interaction network can be represented with an adjacency matrix. The total number of genes was . The value in row and column was 1 if gene and gene had interactions and was 0 if they did not interact.

(1) Normalization. The adjacency matrix will be column-wise normalized

Due to technical limitations, Equation 1 has been placed in the Supplementary Files section.

(2) Iteration. Then, a rank walk step will be iterated. In each round of iteration, the state probabilities at time was based on previous state and the initial state

Due to technical limitations, Equation 2 has been placed in the Supplementary Files section.

where was previous state probabilities at time , was the restart probability and was the initial state probabilities which was a column vector with for the seed genes (NAc, mPFC, PAG neuropathic pain genes, respectively) and to 0 for other genes on the network.

(3) Converge. The iteration process will stop when the difference between two states was smaller than 1×10^{-6}.

After the RWR analysis, each gene on the network will be given a probability of being visited by the seed genes.

The NAc, mPFC and PAG neuropathic pain genes were considered as seed genes, respectively.

To evaluate how significant the probability was, we randomly chose the same number of seed genes 1000 times and calculated the RWR probabilities. If there were more than 50 times that the permutation proverbialities were greater than the actual proverbiality, the permutation p value for that gene was greater than 50/1000=0.05 and that gene will be excluded.

With the permutation p value, we identified the novel neuropathic pain genes in NAc, mPFC and PAG based on RWR analysis. Such expanded NAc, mPFC and PAG neuropathic pain genes can be overlapped to show the cross talk among NAc, mPFC and PAG under neuropathic pain.

Results

The neuropathic pain genes in NAc, mPFC and PAG identified by differential expression analysis

The gene expression profiles of NAc, mPFC and PAG in SNI and Sham mice were analyzed and the differentially expressed genes between SNI and Sham mice in NAc, mPFC and PAG were identified. There were 123, 89 and 795 differentially expressed genes in NAc, mPFC and PAG, respectively.

We compared these three differentially expressed gene lists (**Table S1**) and plotted their Venn Diagram in **Figure 1**. It can be seen that only two genes, Capn11 and Cyp2e1, were overlapped. Obviously, these two genes played important roles in neuropathic pain.

Capn11 (Calpain 11) encodes an intracellular calcium-dependent cysteine protease that has protease activity and calcium-binding capacity [43]. Calpains were reported to participate in some neuronal processes, including synaptic plasticity, neurodegeneration, signal transduction and enhancement [44-46]. The expression of calpains can be observed in several cells types in the central nervous system (CNS), such as spinal cord neurons, cortical neurons, and glial cells [47,48]. According to previous studies, the activities of calpains were markedly increased in neurodegenerative diseases [49], traumatic brain injury [50] and neuropathic pain [51]. Blocking calpain signaling by its inhibitor MDL28170 [52] or silencing calpain-1 levels of the spinal cord [51] attenuates the neuropathic pain and inflammation following peripheral nerve injury [51]. Mahajan et al. investigated that calpain also mediates the editing of AMPA receptor subtypes [53]. Depression is a well-known emotional feature of chronic neuropathic pain. Yossef et al. discovered that chronic pain can increase the AMPA-type glutamate receptor expression levels at the synapses of NAc in a rat model of chronic neuropathic pain with depression-like behaviors [18]. In addition, the increased level of GluA1 leads to the formation of calcium-permeable AMPA receptors (CPARs) and the inhibition of these CPARs in the NAc increases depressive symptoms associated with neuropathic pain [18]. Therefore, CPARs may present a novel therapeutic target for the treatment of depressive symptoms of neuropathic pain.

CYP2E1 (Cytochrome P450 2E1) is a member of the cytochrome P450 superfamily of genes involved in the brain metabolism of ethanol [54-56]. CYP2E1 can be considered as the second enzymatic system in importance for ethanol metabolism in brain [57-59]. This enzyme is widely expressed in various cell types and human brain regions, including the hippocampus, substantia nigra and medulla [60-62]. Further studies are needed to identify the role of Cyp2e1 in chronic pain and sensory symptoms of pain. Toselli et al. found that CYP2E1 was expressed in human AMG and PFC and may influence the drug effects in those regions [63].

As shown above, these two genes functions through complex pathways and regulatory mechanisms, there were many missing genes that facilitate the neuropathic pain responses in different brain regions. To find these hidden genes, we mapped these differentially expressed genes onto functional association network of STRING.

The novel neuropathic pain genes in NAc, mPFC and PAG identified by RWR analysis on the functional association network

To identify more novel neuropathic pain genes in NAc, mPFC and PAG and find their hidden links or cross talks, we mapped the differentially expressed genes onto network and performed RWR analysis on the network. By considering the differentially expressed genes in NAc, mPFC and PAG as seed genes and permuting them 1000 times, we identified the significant novel neuropathic pain genes in NAc, mPFC and PAG with permutating p value smaller than 0.05. There were 623, 888 and 507 novel neuropathic pain genes in NAc, mPFC and PAG, respectively. These novel neuropathic pain genes in NAc, mPFC and PAG were given in **Table S2**.

The Venn Diagram among these novel neuropathic pain genes in NAc, mPFC and PAG on the network was shown in **Figure 2**. There were 25 overlapped genes and they were shown in **Table 1**. These 25 overlapped genes showed great promise in linking the three brain regions and revealing the potential cross talk mechanisms among NAc, mPFC and PAG for neuropathic pain. We will discuss their functions in the next section.

Discussion

Among the 25 genes in **Table 1**, many of them were involved in pathways or functions associated with neuropathic pain genes. The following three genes were most promising.

Asic3 (Acid-sensing ion channels, ASICs) was cationic channel expressed principally in central (CNS) and peripheral (PNS) nervous systems [64,65]. Ion channel modulation is a main approach to achieve novel neuropathic pain management [66]. Evidence from many experiments have suggested the involvement of ASICs in pain sensation [66,67]. Among the ASICs, ASIC3 is known to regulate inflammatory pain, ischemic pain and mechanical pain [64,68]. Inflammation is one of the pain symptoms that induces a significant increase of ASIC3 channel expression in sensory neurons, which demonstrate the crucial role of ASIC3 in the generation of pain associated with inflammation [69]. Therefore, inhibition of ASIC3 channel at the sensory system could obviously help to alleviate pain. In addition, Jeong et al. suggested that ASIC3 may be associated with the antinociceptive effects of amiloride and benzamil, inhibitors for ASIC channels [70,71], in neuropathic pain and blocking ASIC3 channel may be a novel therapeutic strategy in neuropathic pain treatment [72].

Cd200r1 encodes a membrane glycoprotein of the immunoglobulin superfamily that is highly expressed on neurons in the central nervous system while its receptor CD200R is restricted to the surfaces of myeloid lineage cells like macrophages and microglia [73,74]. The CD200-CD200R interaction has been reported to be closely associated with the macrophage-mediated damage in autoimmune disease and various neuroinflammatory diseases [75-78]. Animal models have also shown that loss of immunosuppression through CD200 has significant impact on neuroinflammation and neurodegeneration [79,80]. Hernangomez et al. [80] reported that the CD200/CD200R regulatory system can

suppress the neuroinflammatory reactions associated with peripheral neuropathic pain. CD200/CD200R may be a target for treating neuropathic pain.

MT2 (Metallothioneins II) is a major neuroprotective protein with a high affinity for metals [81,82]. MT2 has been found in many CNS (central nervous system) regions[83,84], such as cortex, hippocampus, brainstem and spinal cord [85]. A series of evidence suggests that metallothioneins (MTs) is essential for the recovery from CNS damage [83,86]. Hidalgo et al. have investigated that MT-I/-II is capable of decreasing inflammatory responses associated with CNS injury and provided credible evidence suggesting that MT-I/-II protects neurons from death [87-89]. Kwon et al. evaluated the expression of MT-I/II in the spinal cord in rat models with inflammatory and neuropathic pain and the results showed that increased MT-I/II participate in the initiation of inflammatory and neuropathic pain [90].

Conclusions

As a common nervous system disease with an incidence of 7-10% in the general population, the mechanisms of neuropathic pain are largely unknown. It is a complex disease involving the structural and functional abnormalities in multiple brain regions. The forebrain, including nucleus accumbens (NAc), medial prefrontal cortex (mPFC) and periaqueductal gray (PAG), all correspond to the response of neuropathic pain. To investigate the molecular mechanism of neuropathic pain across different brain regions, we identified the differentially expressed genes between SNI mice which was a widely used model for neuropathic pain and the Sham mice which was used as control. The differentially expressed genes in NAc, mPFC and PAG were mapped onto STRING network. Using Random Walk with Restart (RWR) analysis, more novel neuropathic pain genes in NAc, mPFC and PAG were revealed based on network structure and more overlapped genes among them had emerged. These overlapped novel neuropathic pain genes in NAc, mPFC and PAG can help us understand how different brain regions communicate with each other and coordinate the regulation of neuropathic pain. These genes worth to be further validated and investigated as therapeutic target.

Abbreviations

NAc: nucleus accumbens

mPFC: medial prefrontal cortex

PAG: periaqueductal gray

SNI: spared nerve injury

RWR: Random Walk with Restart

IASP: International Association for the Study of Pain

PVN: paraventricular nucleus

RVM: rostral ventromedial medulla

RPKM: Reads Per Kilobase per Million mapped reads

GEO: Gene Expression Omnibus

CNS: central nervous system

PNS: peripheral nervous system

CPARs: calcium-permeable AMPA receptors

ASICs: acid-sensing ion channels

MT2: Metallothioneins II

CYP2E1: Cytochrome P450 2E1

Capn11: Calpain 11

Declarations

Ethics approval and consent to participate

This article does not contain any studies with animals and human performed by any of the authors.

Consent to publish

Not applicable.

Availability of data and materials

The data and materials in the current study are available from the corresponding author on reasonable request.

Competing Interests

None.

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Authors' Contributions

Dr LX, XS and XF contributed to the study design. CY conducted the literature search. YM, YQ, FX and LD acquired the data. LX and XS wrote the article. XF performed data analysis. YM drafted. YQ, FX and LD revised the article and gave the final approval of the version to be submitted. All authors read and approved the final manuscript.

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Tables

Table 1 - The 25 overlapped novel neuropathic pain genes in NAc, mPFC and PAG on the network

Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol
Adat2	Ap5b1	Cd200r1	Igdcc3	Prlhr
Adat3	Ap5s1	Cic	Junb	Stoml3
Adgra3	Asic3	Ctu1	Mt1	Ush2a
Adgrv1	Atxn1	Ctu2	Mt2	Vezt
Agt	Cd200	Cyp2e1	Prlh	Whrn

Figures

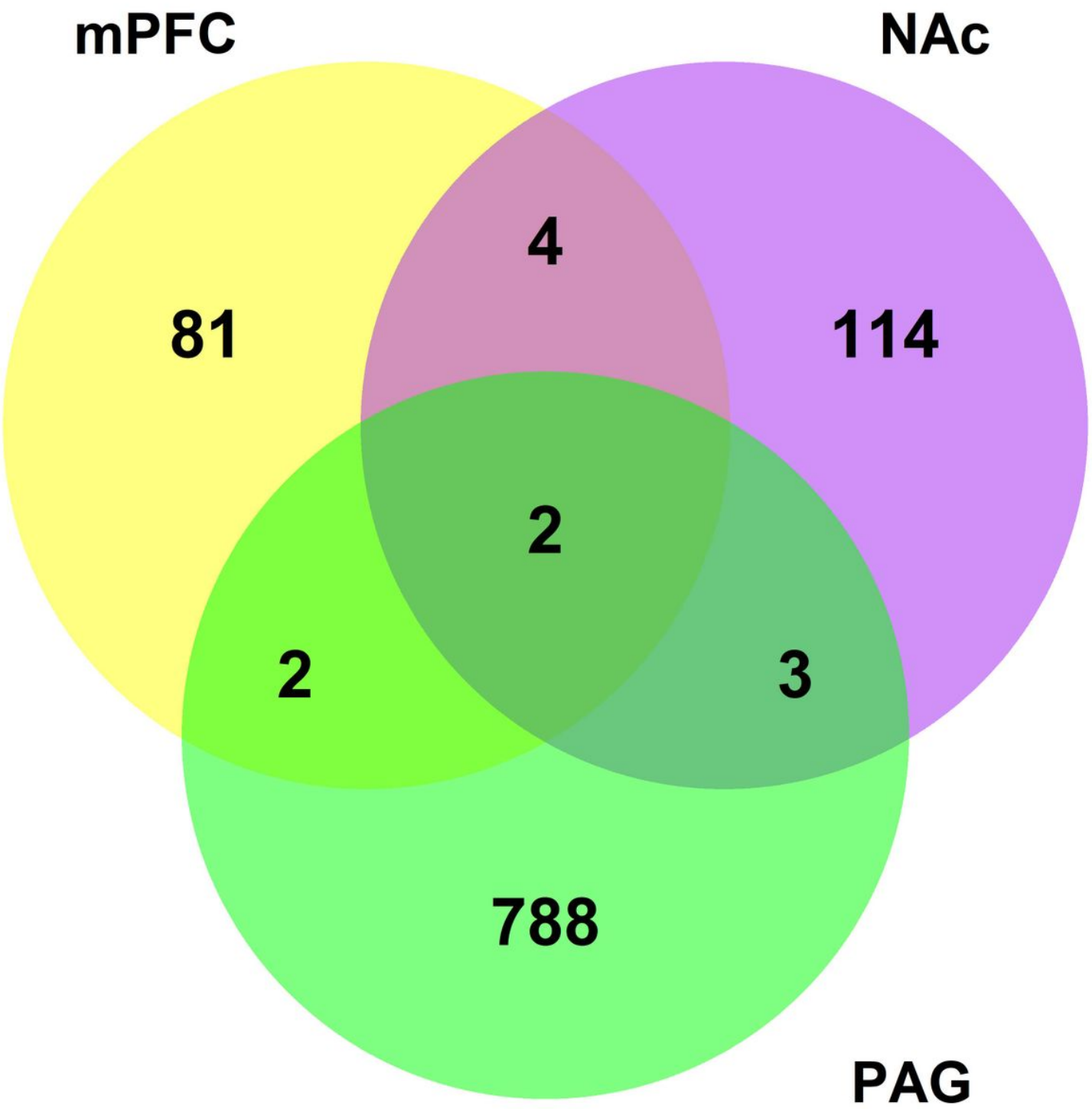

Figure 1

The Venn Diagram of differentially expressed genes in NAc, mPFC and PAG Among the 123, 89 and 795 differentially expressed genes in NAc, mPFC and PAG, only two genes, Capn11 and Cyp2e1, were overlapped. These two genes played important roles in neuropathic pain, but there were many undiscovered neuropathic pain genes in the differentially expression analysis.

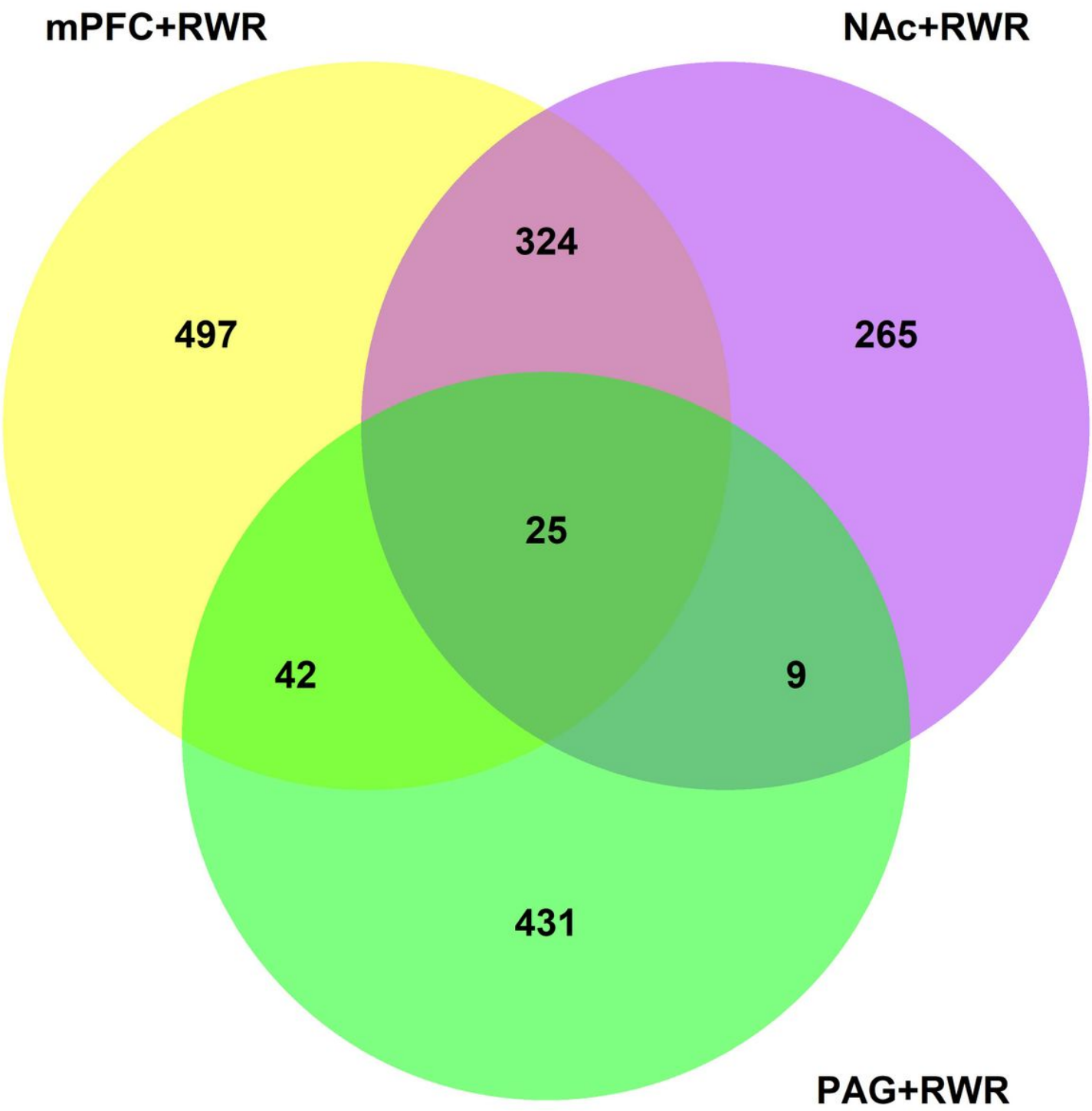

Figure 2

The Venn Diagram of novel neuropathic pain genes in NAc, mPFC and PAG on the network Among the 623, 888 and 507 novel neuropathic pain genes in NAc, mPFC and PAG, 25 genes were overlapped. These genes linked the three brain regions and revealed the potential cross talk mechanisms among NAc, mPFC and PAG for neuropathic pain.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Equation2.png
- TableS1.xlsx
- TableS2.xlsx
- Equation1.png