Bioinformatics analysis of gene and microRNA targets for fibromyalgia

Y. Qiu[1], T.-J. Zhang[1], L.-B. Meng[2], X.-T. Cheng[1], Z. Hua[1]

[1]Anaesthesiology Department, [2]Neurology Department, Beijing Hospital, National Centre of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China.

Abstract

Objective

Fibromyalgia (FM) is the most common chronic pain disease in middle-aged women. Patients may also complain of migraine, irritable bowel syndrome and depression, which seriously affect their work and life, causing huge economic losses to society. However, the pathogenesis of FM is still controversial and the effect of the current treatment is far from satisfactory.

Methods

Differentially expressed genes (DEGs) and miRNAs (DEMs) were found between FM and normal blood samples. The pathway and process enrichment analysis of the genes were performed. Protein-protein interaction network were constructed. Hub genes were found and analysed in The Comparative Toxicogenomics Database.

Results

A total of 102 genes were up-regulated and 46 down-regulated, 206 miRNAs down-regulated, and 15 up-regulated in FM. CD38, GATM, HDC, FOS were found as canditate genes. These genes were significantly associated with musculoskeletal disease, mental disorder, immune system disease. There was partial overlap between metformin therapy-related genes and FM-related genes.

Conclusion

We found DEGs and DEMs in FM patients through bioinformatics analysis, which may be involved in the occurrence and development of FM and serve as potential targets for diagnosis and treatment.

Key words

fibromyalgia, bioinformatics, metformin

Yong Qiu, BA
Teng-jiao Zhang, BA
Ling-bing Meng, BA
Xu-tao Cheng, BA
Zhen Hua, MD

Please address correspondence to:
Zhen Hua,
Anaesthesiology Department,
Beijing Hospital,
National Centre of Gerontology,
Institute of Geriatric Medicine,
Chinese Academy of Medical Sciences,
No. 1 Dahua Road, Dong Dan,
Beijing 100730, P. R. China.
E-mail: huazhenbj528@163.com

Received on December 8, 2019; accepted in revised form on February 4, 2020.

Competing interests: none declared.

Introduction

Fibromyalgia (FM) causes chronic primary pain and fatigue and is the most common cause of chronic generalised musculoskeletal pain (1). Fibromyalgia can occur in children and adults, and the incidence increases with age. What is more, FM is more common in women than in men (2). The prevalence of FM is related to diagnostic criteria. A cross-sectional survey found that the prevalence of FM using the ACR (American College of Rheumatology) 2011 revised diagnostic criteria was 5.4%, compared with 1.2% according to the 2010 criteria and 1.7% based on the 1990 diagnostic criteria (3). However, the detection rate of FM conducted by the US National Health Interview Survey (NHIS) for a sample of 8446 persons, was 1.75%. According to the sample, it was estimated that about 3.94 million people in the United States suffer from FM (4). The missed and delayed diagnosis of a large number of FM sufferers not only affect the effect of treatment, but also increase the medical burden (5). Patients with FM may experience multiple-site pain (MSP), fatigue, and sleep disorders that last for more than 3 months (6). Moreover, 30% to 50% of patients suffer from depression and anxiety. More than 50% of patients suffer from headache, including migraine and tension headache, and seriously reduced quality of life (7). However, the pathogenesis of FM is still controversial. Central pain sensitisation, oxidative stress and mitochondrial dysfunction, abnormal immune system and genetic changes may be involved in the occurrence and development of FM (8-10). In addition, multiple pathogenesis may be involved in the disease progression of the same patient. At the same time, there may be differences in the main mechanisms and inducements of the disease in different patients (6, 11), who may have different complications (1). These evidences suggest the complexity and heterogeneity of the pathogenesis of FM. Commonly used therapeutic drugs include pregabalin, duloxetine, gabapentin and amitriptyline; however, even with the combination of multiple drugs, the therapeutic effect is still far from satisfactory (12, 13). In addition, transcutaneous nerve electrical stimulation-transcutaneous nerve electrical stimulation (transcutaneous electrical nerve stimulation, TENS) can reduce the central excitability and activate the central inhibitory pathway, which may effectively improve the systemic symptoms of FM (14). However, more clinical evidence is needed to prove the effectiveness and safety of TENS. Fibromyalgia still afflicts a large number of patients (15). It is of great significance to further study the pathogenesis of FM and find molecular targets for diagnosis and treatment.

Bioinformatics analysis technology is widely used to find molecular changes in the progression of disease. Liu *et al*. found several differentially expressed genes (DEGs) and miRNAs (DEMs) by sequencing synovium samples of patients with rheumatoid arthritis (RA). Further verification and analysis showed that miR-5571-3p and miR-135B-5p may participate in the occurrence and development of RA by regulating immune activity and inflammation-related pathways (16). In addition, Wang *et al*. found a number of molecules related to neuropathic pain after spinal cord injury through bioinformatics analysis. Further analysis found that mir-204-5p may be involved in the occurrence and development of neuropathic pain after spinal cord injury by regulating protein modification and central nervous system biological processes. This suggests that related molecules may be used as diagnostic and therapeutic targets (17). What is more, some studies have identified several genes and miRNA molecules that may be used for early diagnosis of FM by sequencing blood samples from patients with FM (18, 19). However, there are still few reports on the use of bioinformatics analysis to explore the pathogenesis of FM.

After analysing the peripheral blood sequencing data of FM patients, we found several DEGs and DEMs. Then we analysed DEGs by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and constructed protein-protein interaction (PPI). The hub gene that may be involved

in the progression of FM were found. What is more, the possible targets of pregabalin, milnacipran, amitriptyline in the treatment of FM were analysed. In addition, the possibility of using metformin in the treatment of FM was explored. Finally, we made a preliminary analysis of the role of candidate genes in FM.

Materials and methods

Dataset

The GEO (http://www.ncbi.nlm.nih.gov/geo) is a platform for retrieving gene expression data (20). One expression profiling dataset [GSE67311 (GPL11532 platform)] and one microRNA expression profiling dataset [GSE65033 (GPL14903 platform)] were respectively downloaded from the GEO database. The GSE67311 dataset includes gene expression in the whole blood of 70 FM patients and 70 healthy matched controls. We chose only 61 FM blood samples and 68 normal blood samples from the dataset based on the source. The GSE65033 dataset includes miRNA expression in PBMCs (Peripheral Blood Mononuclear Cells) of 11 FM patients and 10 healthy controls.

DEGs identification

GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) is an online tool based on GEOquery and limma R packages, used to identify DEGs in datasets from the GEO (21). GEO2R may also be used to distinguish DEGs and DEMs between FM and normal blood samples. The rule of statistical significance is that p-value<0.05 and Fold change (FC)>0.2 or FC<-0.2 (GSE67311), Fold change (FC)>1 or FC<-1 (GSE65033). Volcano diagrams were delineated by SangerBox software based on R language(http://sangerbox.com/).

DEGs annotation

The Database for Annotation, Visualisation and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/home.jsp; v. 6.8) is an online tool with an integrated discovery and annotation function (22). To perform the biological process (BP), cellular component (CC), molecular function (MF) and KEGG analysis of DEGs, DAVID online tool was implemented. The rule of statistical significance is that p<0.05. In addition, the pathway and process enrichment analysis were performed by the Metascape (http://metascape.org/gp/index.html), a powerful annotation analysis tool for gene function.

Protein-protein interaction (PPI) network construction

The Search Tool for the Retrieval of Interacting Genes (STRING) (http://string.embl.de/) was used to construct a PPI network of the identified DEGs. In addition, Cytoscape visualisation software v. 3.6.1 was used to present the network (23). The most significant module in the PPI network was identified by Molecular Complex Detection tool (MCODE), a pug-in of Cytoscape. The criteria was that MCODE scores >2, the degree of cut-off=2, node score cut-off=0.2, k-score=2 and maximum depth=100.

Identification and analysis of miRNA target genes

MiRNet (http://www.mirnet.ca) includes a large number of data on the interaction between miRNA and genes. It can effectively predict the downstream target genes of miRNA (24). Target genes of the top 10 up-regulated and down-regulated miRNAs were predicted by miRNet. After that, pathway and process enrichment analysis of the miRNA target genes were performed by Metascape.

Hub genes identification

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) can effectively predict the correlation among diseases, drugs and genes. CTD can calculate the inference scores and provide ideas for exploring the molecular mechanism of the disease and searching for therapeutic drugs (25). The target genes predicted by miRNA intersected with DEGs were found. Also, the CTD database provides genes related to FM. The intersection genes of DEGs, miRNA target genes and FM were found. Common genes were analysed by DAVID and shown by bubble diagrams. In addition, common genes were analysed by Cytoscape. The key modules were found after MCODE analysis in PPI. These module genes and the intersection genes of DEGs and miRNA target genes were hub genes.

CTD analysis and candidate genes identification

Candidate genes were found from FM-related genes, FM drug-related genes, miRNA target genes, DEGs crossover analysis of by Funrich software (http://funrich.org/). What is more, CD38, GATM, HDC, FOS were found as canditate genes. The association of these genes with musculoskeletal disease (MSD), mental disorder (MD), immune system disease (ISD) were indicated by inferce scores provided by CTD. In addition, Brain RNA-seq is an RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html)(26). The expression and localisation of candidate genes in brain cells were analysed by Brain RNA-seq.

TFs (Transcription factors) predicted

TransmiR v. 2.0 is a updated transcription factor-microRNA regulation database (http://www.cuilab.cn/transmir) (27). Candidate miRNAs related transcription factors were predicted by TransmiR v. 2.0. Furthermore, the schematic diagram of part of the miRNA-mRNA network were shown by cytoscape.

Ethics approval and consent to participate

The data of this research was downloaded from the GEO database, a public website, and all institutional and national guidelines for the care and use of participates were followed.

Results

Screening of DEGs and DEMs between fibromyalgia and normal control samples

Volcano diagrams showed the DEGs in the GSE67311. A total of 102 genes were up-regulated, 46 down-regulated in FM (Fig. 1A). DEMs were shown in Figure 1B with a total of 206 miRNAs down-regulated, 15 up-regulated in FM in the GSE65033.

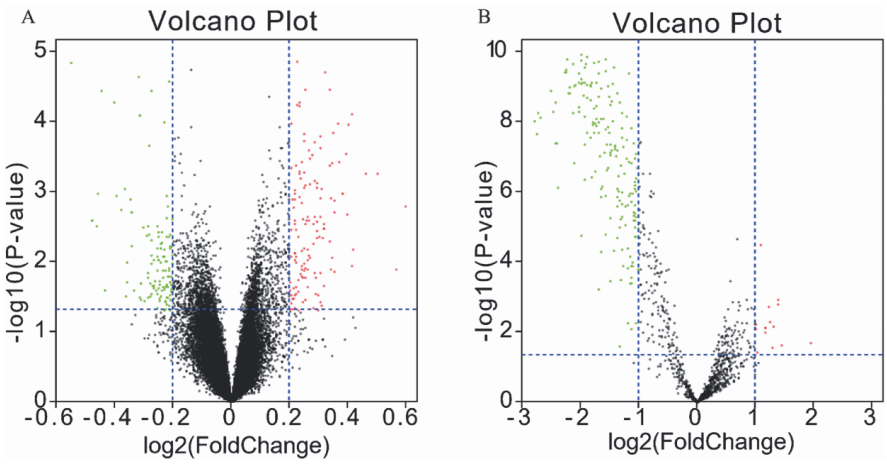

Fig. 1. Identification of DEGs and DEMs between fibromyalgia and normal blood samples. **A**: The DEGs between fibromyalgia and normal blood samples in the GSE67311 were presented in the volcano plots, in which the green nodes mean the down-regulated DEGs, and the red nodes mean the up-regulated DEGs; **B**: The DEMs between fibromyalgia and normal blood samples in the GSE65033 were presented in the volcano plots.

Table I. GO and KEGG pathway enrichment analysis of DEGs in fibromyalgia samples.

Term	Description	Count in gene set	*p*-value
GO:0042592	homeostatic process	12	0.00853
GO:0006508	proteolysis	12	0.07451
GO:0044265	cellular macromolecule catabolic process	10	0.04298
GO:0009057	macromolecule catabolic process	10	0.06313
GO:0050801	ion homeostasis	7	0.04799
GO:0002520	immune system development	6	0.03253
GO:0005886	plasma membrane	31	0.07698
GO:0044459	plasma membrane part	22	0.02760
GO:0005856	cytoskeleton	14	0.08688
GO:0031226	intrinsic to plasma membrane	13	0.0745
GO:0004842	ubiquitin-protein ligase activity	4	0.06988
GO:0019787	small conjugating protein ligase activity	4	0.09263
GO:0005200	structural constituent of cytoskeleton	3	0.08288
GO:0019865	immunoglobulin binding	2	0.09282
hsa00860	Porphyrin and chlorophyll metabolism	3	0.01586

GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; DEGs: differentially expressed genes.

Functional annotation for DEGs using KEGG and GO analysis

The results of GO analysis revealed that variations were predominantly enriched in homeostatic process, cellular macromolecule catabolic process, ion homeostasis, immune system development, small conjugating protein ligase activity (Table I). KEGG analysis demonstrated that DEGs were largely enriched in porphyrin and chlorophyll metabolism (Table I). Pathway and process enrichment analysis by Metascape were shown in Figure 2A-B-C.

Construction of the PPI network

Construction of a PPI network revealed 176 edges and 76 nodes in the PPI network (Fig. 3A). The network possessed significantly more interactions than expected, suggesting that the identified proteins are at least partially associated. The key modules of MCODE analysis were shown in Figure 3C.

Target genes of miRNA

The top 5 up-regulated and down-regulated miRNAs in FM samples were shown in Table II. In addition, the prediction of miRNA target genes by miRNet showed that there were some target genes in up-regulated miRNA hsa-mir-19a-5p, down-regulated miRNA hsa-mir-126-5p and hsa-mir-223-3p (Fig. 3B). Pathway and process enrichment analysis of the target genes were shown in Figure 2D-E-F.

Hub gene selection and analysis

MCODE key modules of the DEGs were shown in Figure 3C. GATM, TAL1, CARM1, CD38, ZNF138 were common genes of miRNA target genes and DEGs (Fig. 3D). In addition, after analysing the intersection of FM-related genes and DEGs, miRNA target genes, a total of 180 genes were found (Fig. 4A). DAVID analysis results were shown in Figure 4B-E. PPI were shown in Figure S1 A. Key modules were shown in Figure S1 B-F. Together with GATM, TAL1, CARM1, CD38, ZNF138, these module genes were defined as hub genes.

CTD analysis and candidate genes identification

Pregabalin, milnacipran, amitriptyline, celecoxib and venlafaxine related genes were obtained from CTD database. Furthermore, drug-related genes, FM-related genes and DEGs, miRNA target genes common genes were found (Fig. S2 A1-F2). The FOS gene may be involved in the treatment of FM with pregabalin (Fig. S2 A2), while the HDC gene may be involved in the treatment of FM with milnacipran (Fig. S2 B1), and the NLRP3 gene may be involved in the treatment of FM with amitriptyline (Fig. S2 C2). In addition, there were some common genes among metformin-related genes, FM-related genes and DEGs or miRNA target genes. Furthermore, the common genes included some hub genes, suggesting that metformin may be useful in the treatment of FM (Fig. S2 F1-F2). At the same time, candidate genes (CD38, GATM, HDC, FOS) were mainly related to rheumatoid RA, musculoskeletal abnormalities, muscle weakness, osteoporosis, sleep wake disorders, mental disorders, anxiety disorders, depressive disorder, immunologic deficiency, hypersensitivity (Fig. 5A-D). What is more, CD38 and FOS in the brain were mainly located in astrocyte, and GATM and HDC were mainly in microglia (Fig. 5E).

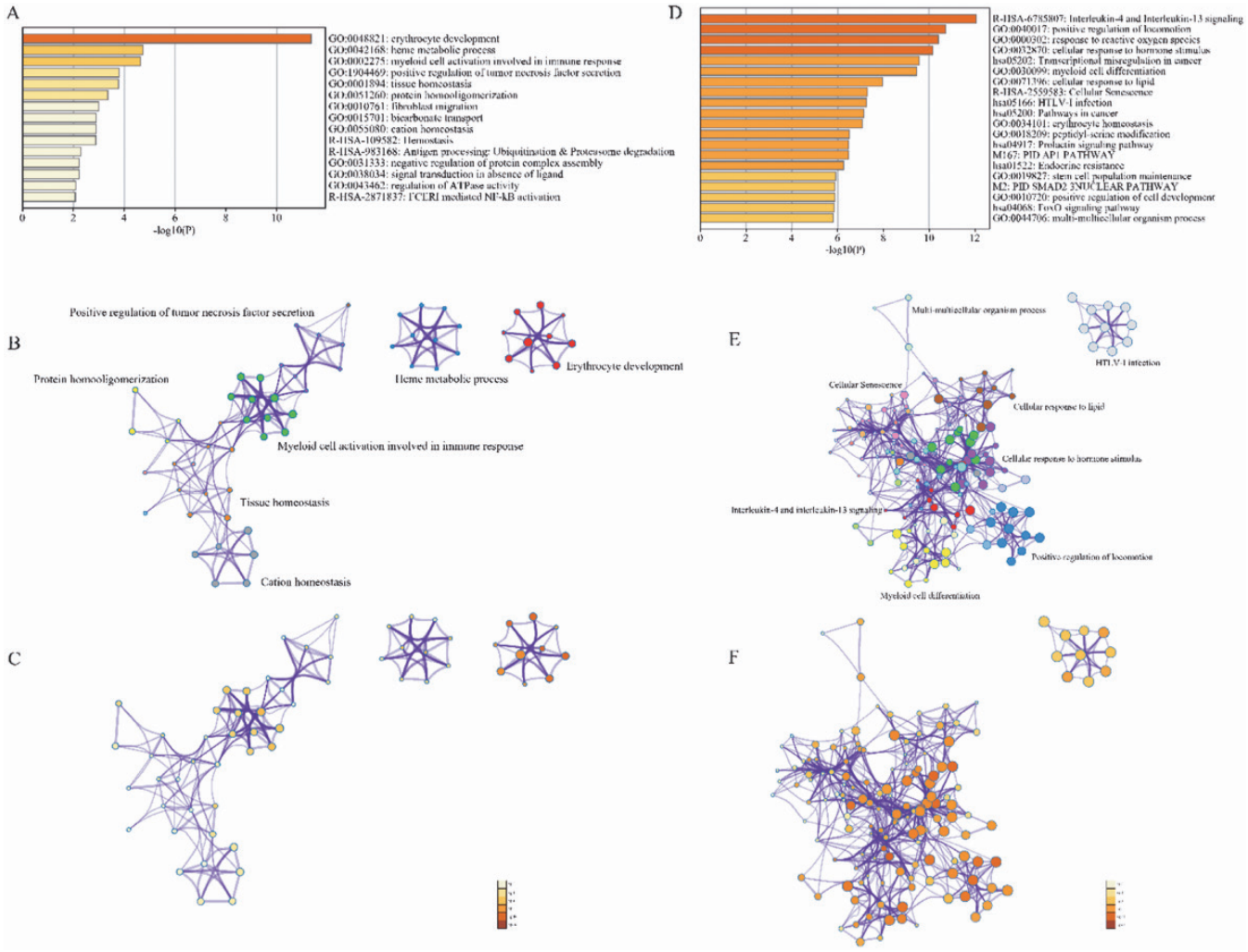

Fig. 2. The enrichment analysis of DEGs and miRNA target genes by DAVID and Metascape.
A: Bar graph of enriched terms across DEGs, coloured by *p*-values; **B**: Network of enriched terms, coloured by cluster; **C**: Network of enriched terms, coloured by significant *p*-value; **D**: Bar graph of enriched terms across miRNA target genes, coloured by *p*-values; **E**: Network of enriched terms, coloured by cluster; **F**: Network of enriched terms, coloured by significant *p*-value.

TFs predicted

Up-regulated miRNA hsa-mir-19a-5p and down-regulated miRNA hsa-mir-223-3p, hsa-mir-126-5p related TFs were predicted in Figure 6A. At the same time, the schematic diagrams of some miRNA-mRNA network are shown in Figure 6B.

Discussion

Fibromyalgia is the most common chronic pain disease in middle-aged women. In addition to widespread pain and sleep disorders, patients may also complain of migraine, irritable bowel syndrome and depression. These symptoms seriously affect the patients' work and life, which then cause huge economic losses to society (2, 6). In addition, after the revision of the diagnostic criteria by the ACR, the morbidity rate of the population is about 5.4%. However, the detection rate of FM is 1.75%, suggesting that a large number of patients do not get correct and timely diagnosis and treatment (3, 4). The main reason for this phenomenon is that the mechanism of FM is unclear and there are no specific diagnostic markers. As a result, the treatment method and effect are far from satisfactory (28, 29). Therefore, it is of great value to find the molecular mechanism of FM in order to make the early diagnosis and specific treatment of FM. In this study, by analysing the peripheral blood and monocyte sequencing data of patients with FM, we found a number of genes and miRNAs that may be involved in the occurrence and development of FM. In addition, the possible mechanism of the involvement of CD38, HDC and FOS in FM were discussed. Furthermore, the feasibility of metformin in the treatment of FM was analysed.

CD38 (cluster of differentiation 38 molecule) is a transmembrane glycoprotein, mainly involved in the regulation of NAD+ nucleosidase activity, identical protein binding, response to hypoxia, positive regulation of cytosolic calcium ion concentration, apoptotic signalling pathway and positive regulation of B cell proliferation (30).What is more, The abnormality of CD38 gene single nucleotide polymorphism (SNP) is associated with depression and other psychiatric symptoms (31). Ning *et al.* found abnormal expression of CD38 in patients with inflammatory bowel dis-

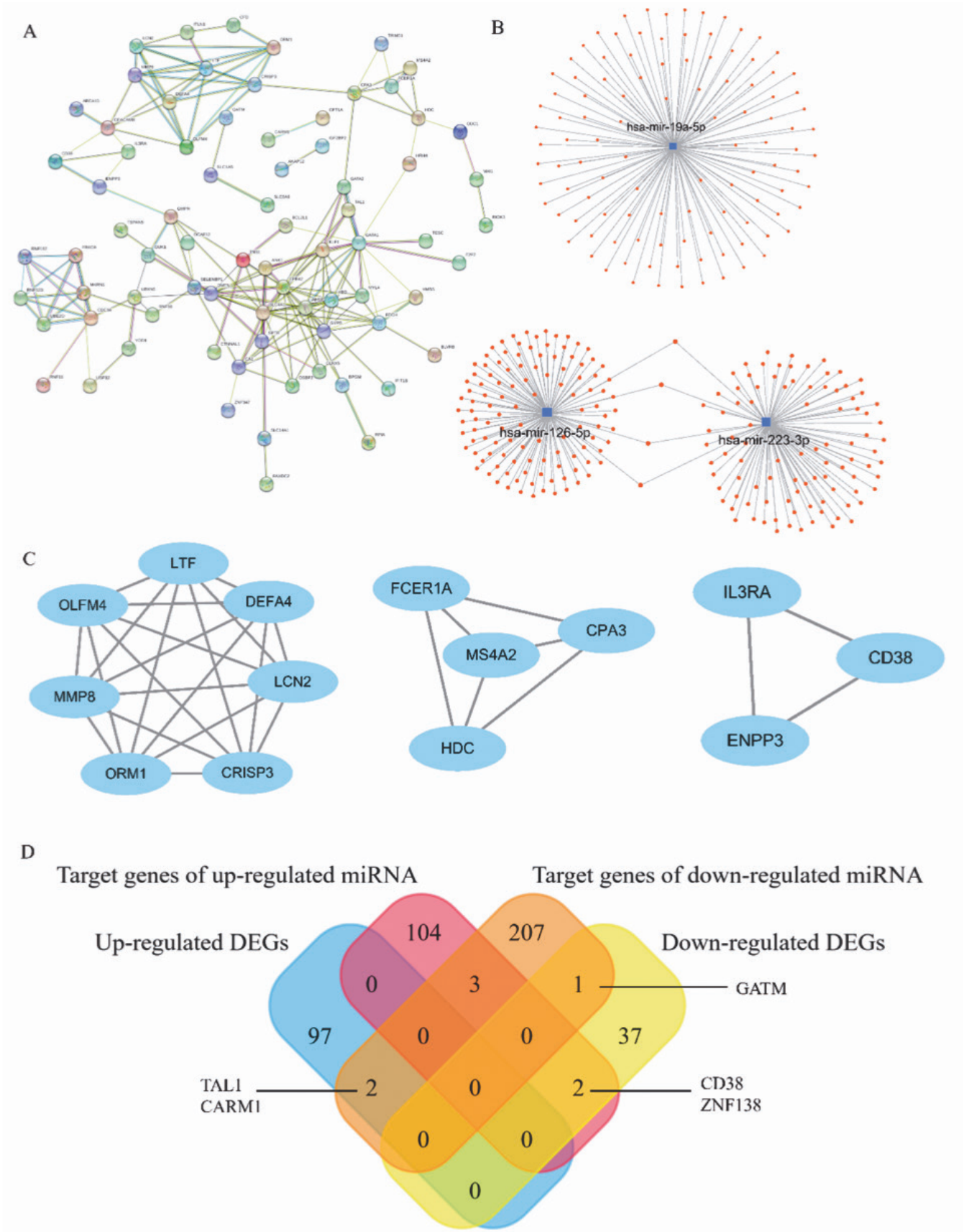

Fig. 3. Protein-protein interaction (PPI) network and common genes. **A**: Protein-protein interaction network of DEGs; **B**: Target genes in up-regulated miRNA hsa-mir-19a-5p, down-regulated miRNA hsa-mir-126-5p and hsa-mir-223-3p; **C**: Key modules of the DEGs; **D**: GATM, TAL1, CARM1, CD38, ZNF138 were common genes of miRNA target genes and DEGs.

Table II. The top 5 up-regulated and down-regulated miRNAs in fibromyalgia samples.

MiRNA	Change	LogFC	*p*-value
Hsa-mir-367	Up-regulated	1.94	2.13E-02
Hsa-mir-646	Up-regulated	1.45	2.51E-02
Hsa-mir-876-3p	Up-regulated	1.39	1.22E-03
Hsa-mir-548z	Up-regulated	1.38	1.61E-03
Hsa-mir-3164	Up-regulated	1.31	6.82E-03
Hsa-mir-148a	Down-regulated	-3.03	8.26E-11
Hsa-mir-338-3p	Down-regulated	-3.49	8.98E-12
Hsa-mir-451	Down-regulated	-3.67	3.98E-08
Hsa-mir-145	Down-regulated	-3.88	2.80E-09
Hsa-mir-143	Down-regulated	-3.99	1.80E-09

FC: fold change.

ease (IBD) by proteomic analysis. Further analysis suggests that CD38 may participate in the progression of IBD through the nicotinamide adenine dinucleotide (NAD) metabolism and signalling pathway, suggesting that CD38 may be a therapeutic target (32). In addition, Sadeghi *et al*. found that phenytoin may inhibit Ca2+ influx through regulate cyclase activity of CD38 and cADPR (cyclic adenosine diphosphate ribose), and then play an anticonvulsant role in the treatment of depression (33). Hull *et al*. found that CD38 may be involved in morphine tolerance and pain receptor abnormalities by regulating CD38-cADPR-ryanodine receptor Ca (2+) signalling pathway (34). In fact, voltage-gated calcium (Cav) channels (VGCC) participates in a variety of physiological activities. Zhao *et al*. found that abnormal activity of T-type, a calcium channel, is significantly associated with epilepsy, mental disorders and pain (35). Therefore, calcium channel-related signalling pathways may also be involved in the pathogenesis of FM. In addition, Roboon found that knockout CD38 could inhibit neuroinflammation and glial cell activation, suggesting that CD38 could be used as a target for reducing neuroinflammation and glial cell activation (36). Our analysis shows that CD38 is mainly enriched in Astrocyte. Similar to the above study, we found that FM patients with low expression of CD38, and CD38 is the target gene of up-regulated miRNA hsa-mir-19a-5p. We speculate that CD38 may be involved in the occurrence and development of FM by regulating calcium-related signal pathways, regulating inflammatory response, regulating apoptosis and other mechanisms. Furthermore, CD38 may be a therapeutic target for improving pain, depression and IBD symptoms in FM. The molecular mechanism of the involvement of CD38 in FM is worthy of further exploration.

HDC (histidine decarboxylase) is mainly involved in histidine decarboxylase activity, protein binding, catecholamine biosynthetic process. What is more, histamine participates in pain modulation. Yu *et al*. found that central histamine may participate in central sensitisation and induce neuropathic pain (37). In addition, Jin *et al*. found the abnormal expression of HDC gene by sequencing the whole blood genome of patients with Complex regional pain syndrome (CRPS), suggesting that HDC and other related molecules may be used as therapeutic targets for CRPS (38). Furthermore, Munari *et al*. found that the abnormality of brain histamine system may be involved in the occurrence and development of depression (39). We found low expression of HDC gene in FM patients by bioinformatics analysis. Moreover, HDC may also be the target of milnacipran in the treatment of FM. We speculate that HDC participates in the progression of FM through immune regulation and central pain regulation. The related molecular mechanism is worthy of further exploration.

FOS (Fos proto-oncogene, AP-1 transcription factor subunit) is mainly involved DNA-binding transcription factor activity, DNA methylation, inflammatory response, nervous system development, sleep and cellular response to calcium ion. What is more, Sun *et al*. found that there is interaction between miR-221, lincRNA p21 and FOS. Overexpression of lincRNA p21 promotes fos expression by regulating miR-221 node, which inhibits hippocampal neuronal apoptosis in diabetic mice, suggesting that related molecules may be used as targets for the treatment of diabetes (40). Touj *et al*. found that c-Fos immunoreactivity may be involved in pain hypersensitivity through the detection and analysis of mechanical and thermal pain thresholds and pain-related molecules in the animal model of blind mice (41). In addition, Borysovych *et al*. found that FOS-related Fos-immunoreactive nuclei is involved in the regulation of cortical spreading depression (CSD) and activation of on neuronal activation in the periaqueductal grey matter (PAG), suggesting that FOS may be involved in central sensitisation-induced migraine (42). Furthermore, Nascimento *et al*. found β-cyclodextrin produce anti-hyperalgesic activity and increase FOS protein expression, in the mouse model of FM, suggesting that FOS may participate in the central sensitisation of FM and act as a therapeutic target (43). Consistent with the above studies, we found that FOS is a target gene for abnormally up-regulated miRNA hsa-mir-19a-5p in patients with FM. FOS may also be a target for pregabalin in the treatment of FM. We speculate that FOS participates in the progression of FM by regulating immunity, regulating inflammation and inducing central sensitisation. The related molecular mechanism is worthy of further study.

Metformin is a commonly used drug to control blood sugar in patients with diabetes. At the same time, it has cardiovascular protective effect, which can reduce mortality in patients with coronary heart disease and heart failure (44). What is more, metformin may also delay aging and inhibit tumour growth (45). In addition, metformin regulates AMPK (Adenosine 5'-monophosphate activated protein kinase), inhibits mitochondrial glycerol-3-phosphate dehydrogenase and affects cell oxidation, and regulates intestinal flora (46). Furthermore, metformin can stimulate specific aging neural stem cells to produce differentiation, which in turn promotes the regeneration of nerve myelin

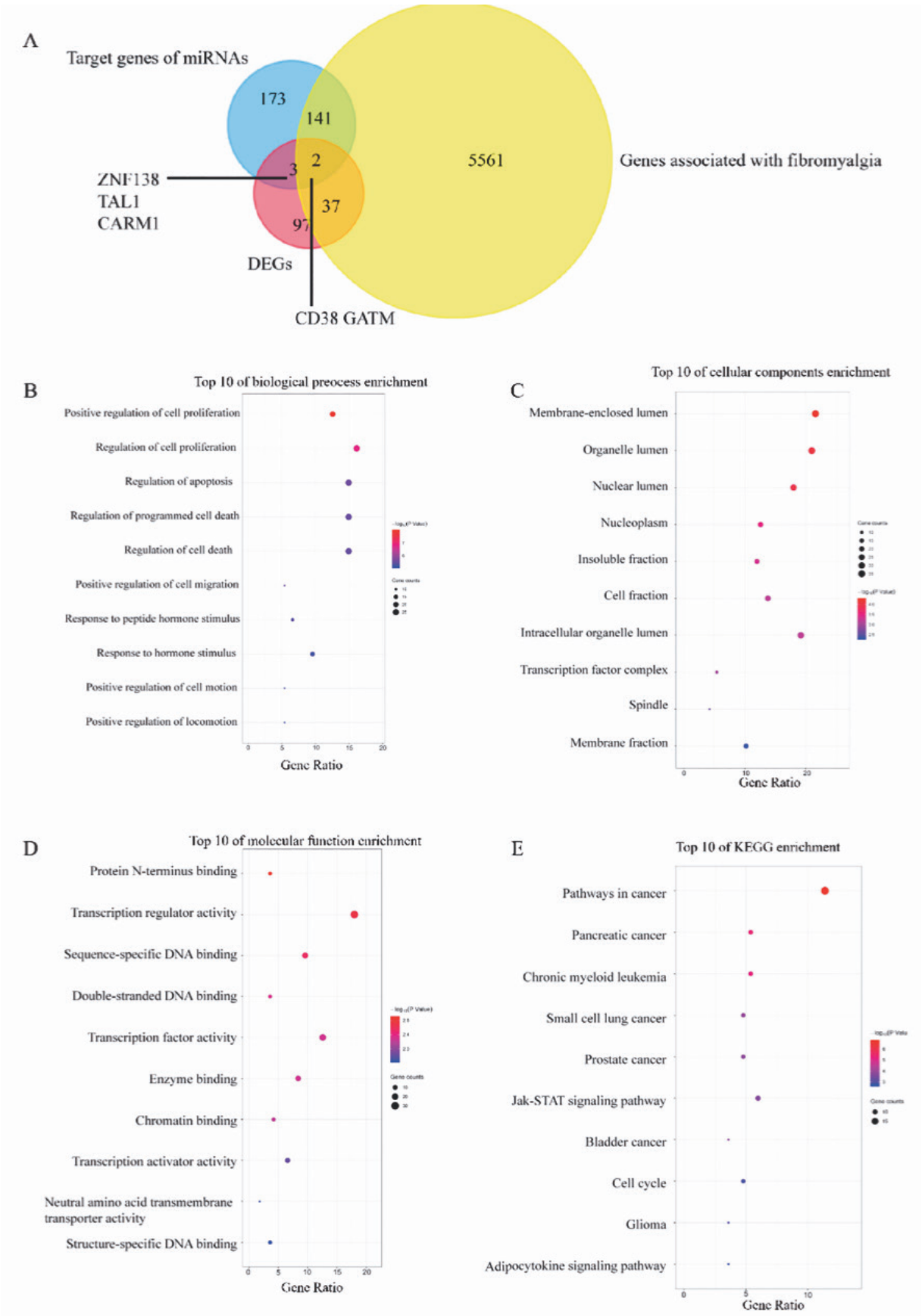

Fig. 4. Common genes of DEGs, miRNA target genes and fibromyalgia-related genes. **A**: There were 180 genes overlap between the genes calculated by bioinformatics analysis and FM-related genes. (**B**-**E**) GO and KEGG analysis of the common genes by DAVID.

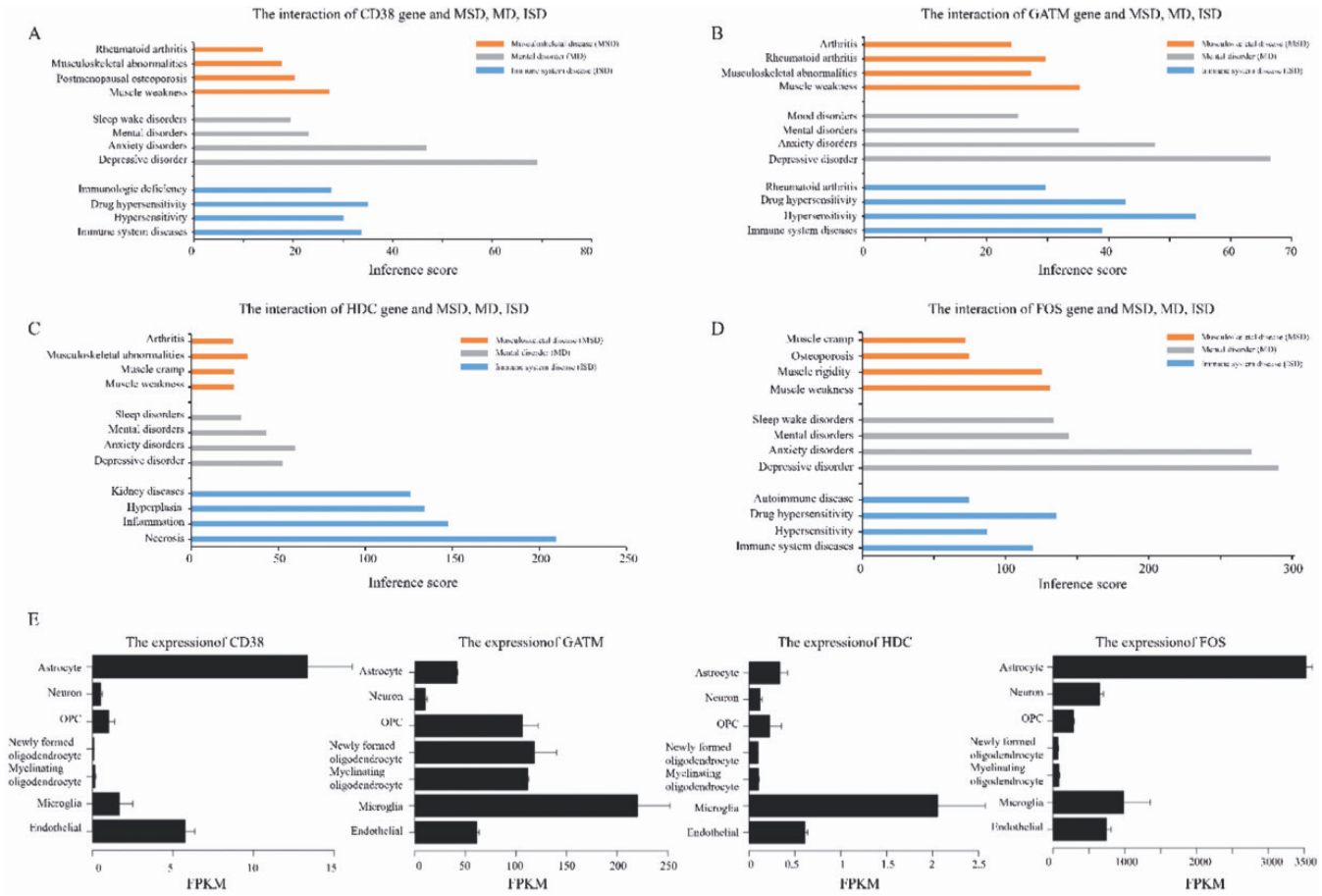

Fig. 5. Candidate genes analysis in CTD and expression, localisation in brain cells.
A: CD38, **B**: GATM, **C**: HDC, **D**: FOS, **E**: The expression and localisation of candidate genes in brain cells.

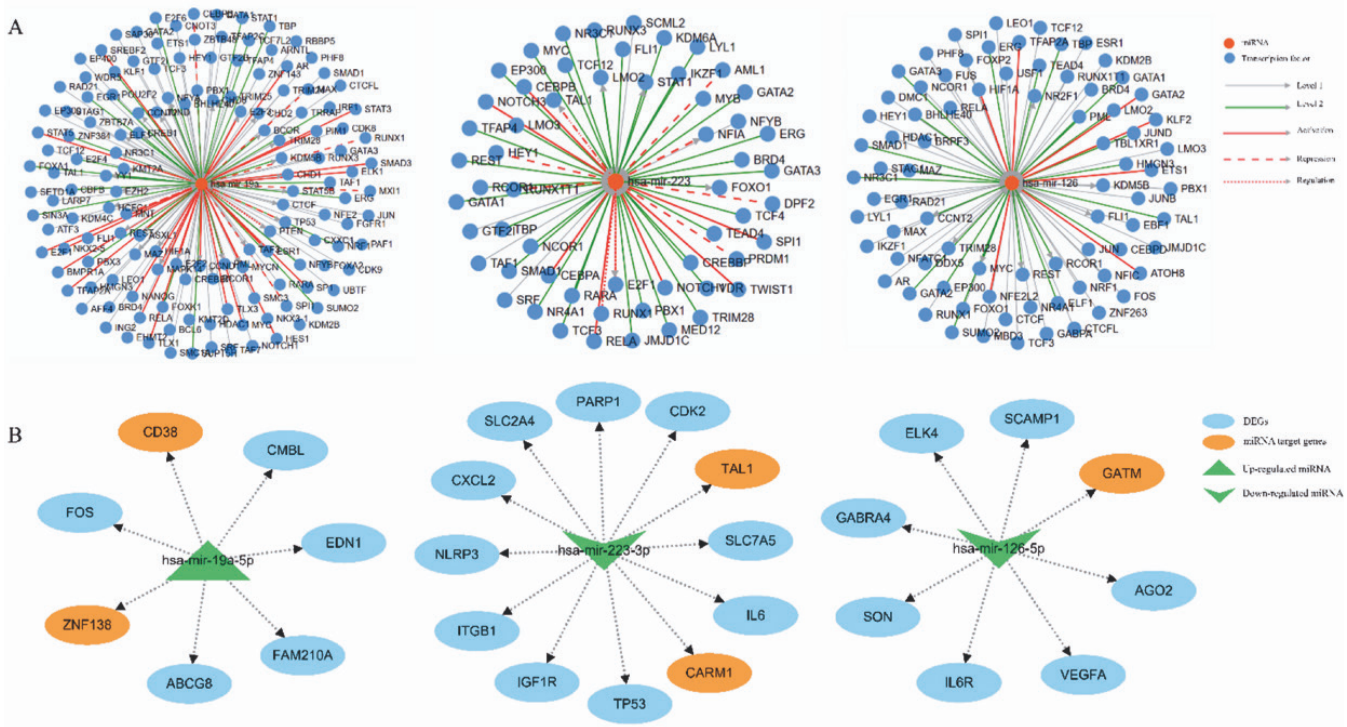

Fig. 6. Transcription factors of miRNAs predicted and miRNA-mRNA network. **A**: Up-regulated miRNA hsa-mir-19a-5p and down-regulated miRNA hsa-mir-223-3p, hsa-mir-126-5p related TFs. **B**: Diagrams of part of the miRNA-mRNA network.

sheath (47). Alcocer *et al*. found that metformin may improve mitochondrial dysfunction and chronic pain symptoms in patients with FM through the AMPK pathway (48). At the same time, AMPK activity and pyrin domain containing 3 (NLRP3) inflammasome activation are associated with several metabolic and inflammatory diseases. Metformin may regulate the axis of inflammation to improve pain, fatigue and other symptoms in patients with FM (49). Consistent with the above study, we found that there are many common related genes between metformin and FM. We speculate that metformin may be used in the treatment of FM by regulating inflammation and central sensitisation. The related molecular mechanism needs to be explored.

Although this study has carried out rigorous bioinformatics analysis, there are still some shortcomings. Firstly, the sample size of the datasets are small. Larger sample size sequencing data is needed to make the conclusion more accurate. Secondly, there is neither sample verification nor self-sequencing verification in this paper. Comprehensive verification is needed in animal experiments and clinical trials in order to have a better understanding of the molecular mechanism of the occurrence and development of FM. Thirdly, we predicted the miRNA-related transcription factors and the possible molecular targets of drug action, as well as the possibility of metformin in the treatment of FM, but these hypotheses need to be verified by further experiments.

Conclusion

We found DEGs and DEMs in FM patients through bioinformatics analysis. These molecules may be involved in the occurrence and development of FM and serve as potential targets for diagnosis and treatment. Bioinformatics analysis can help us to explore the molecular mechanism of FM and find possible therapeutic drugs.

Acknowledgements

The authors would like to thank Menglei Hao for her statistical assistance and suggestions during the submitting process.

References

1. RAHMAN A, UNDERWOOD M, CARNES D: Fibromyalgia. *BMJ* 2014; 348: g1224.
2. JONES GT, ATZENI F, BEASLEY M, FLUSS E, SARZI-PUTTINI P, MACFARLANE GJ: The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. *Arthritis Rheumatol* 2015; 67: 568-75.
3. WOLFE F, CLAUW DJ, FITZCHARLES MA *et al*.: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* (Hoboken) 2010; 62: 600-10.
4. WALITT B, NAHIN RL, KATZ RS, BERGMAN MJ, WOLFE F: The prevalence and characteristics of fibromyalgia in the 2012 National Health Interview Survey. *PLoS One* 2015; 10: e0138024.
5. KASHIKAR-ZUCK S, CUNNINGHAM N, PEUGH J *et al*.: Long-term outcomes of adolescents with juvenile-onset fibromyalgia into adulthood and impact of depressive symptoms on functioning over time. *Pain* 2019; 160: 433-41.
6. CLAUW DJ: Fibromyalgia: a clinical review. *JAMA* 2014; 311: 1547-55.
7. KUCUKSEN S, GENC E, YILMAZ H *et al*.: The prevalence of fibromyalgia and its relation with headache characteristics in episodic migraine. *Clin Rheumatol* 2013; 32: 983-90.
8. SARZI-PUTTINI P, ATZENI F, MEASE PJ: Chronic widespread pain: from peripheral to central evolution. *Best Pract Res Clin Rheumatol* 2011; 25: 133-9.
9. CORDERO MD, DE MIGUEL M, CARMONA-LOPEZ I, BONAL P, CAMPA F, MORENO-FERNANDEZ AM: Oxidative stress and mitochondrial dysfunction in fibromyalgia. *Neuro Endocrinol Lett* 2010; 31: 169-73.
10. RYABKOVA VA, CHURILOV LP, SHOENFELD Y: Neuroimmunology: what role for autoimmunity, neuroinflammation, and small fiber neuropathy in fibromyalgia, chronic fatigue syndrome, and adverse events after human papillomavirus vaccination. *Int J Mol Sci* 2019; 18: 20.
11. FONT GT, BORDOY FC, JUAN MA *et al*.: Prevalence of fibromyalgia and associated factors in Spain. *Clin Exp Rheumatol* 2020; [Epub ahead of print].
12. GILRON I, CHAPARRO LE, TU D *et al*.: Combination of pregabalin with duloxetine for fibromyalgia: a randomized controlled trial. *Pain* 2016; 157: 1532-40.
13. THORPE J, SHUM B, MOORE RA, WIFFEN PJ, GILRON I: Combination pharmacotherapy for the treatment of fibromyalgia in adults. *Cochrane Database Syst Rev* 2018; 2: CD010585.
14. DAILEY DL, VANCE CG, RAKEL BA *et al*.: A randomized controlled trial of TENS for movement-evoked pain in women with fibromyalgia. *Arthritis Rheumatol* 2019; [Epub ahead of print].
15. GENDELMAN O, AMITAL H, BAR-ON Y *et al*.: Time to diagnosis of fibromyalgia and factors associated with delayed diagnosis in primary care. *Best Pract Res Clin Rheumatol* 2018; 32: 489-99.
16. LIU C, PAN A, CHEN X *et al*.: MiR-5571-3p and miR-135b-5p, derived from analyses of microRNA profile sequencing, correlate with increased disease risk and activity of rheumatoid arthritis. *Clin Rheumatol* 2019; 38: 1753-65.
17. WANG Y, YE F, HUANG C *et al*.: Bioinformatic analysis of potential biomarkers for spinal cord-injured patients with intractable neuropathic pain. *Clin J Pain* 2018; 34: 825-30.
18. JONES KD, GELBART T, WHISENANT TC *et al*.: Genome-wide expression profiling in the peripheral blood of patients with fibromyalgia. *Clin Exp Rheumatol* 2016; 34 (Suppl. 96): S89-98.
19. CERDA-OLMEDO G, MENA-DURAN AV, MONSALVE V, OLTRA E: Identification of a microRNA signature for the diagnosis of fibromyalgia. *PLoS One* 2015; 10: e0121903.
20. WANG Z, MONTEIRO CD, JAGODNIK KM *et al*.: Extraction and analysis of signatures from the Gene Expression Omnibus by the crowd. *Nat Commun* 2016;. 7: 12846.
21. BARRETT T, WILHITE SE, LEDOUX P *et al*.: NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res* 2013; 41 (Database issue): D991-5.
22. HUANG DW, SHERMAN BT, TAN Q *et al*.: The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol* 2007; 8: R183.
23. SMOOT ME, ONO K, RUSCHEINSKI J, WANG PL, IDEKER T: Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 2011; 27: 431-2.
24. FAN Y, SIKLENKA K, ARORA SK, RIBEIRO P, KIMMINS S, XIA J: miRNet - dissecting miRNA-target interactions and functional associations through network-based visual analysis. *Nucleic Acids Res* 2016; 44(W1): W135-41.
25. DAVIS AP, GRONDIN CJ, JOHNSON RJ *et al*.: The Comparative Toxicogenomics Database: update 2019. *Nucleic Acids Res* 2019; 47(D1): D948-D954.
26. ZHANG Y, CHEN K, SLOAN SA *et al*.: An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* 2014; 34: 11929-47.
27. TONG Z, CUI Q, WANG J, ZHOU Y: TransmiR v2.0: an updated transcription factor-microRNA regulation database. *Nucleic Acids Res* 2019; 47(D1): D253-D258.
28. GOLDENBERG DL, CLAUW DJ, PALMER RE, CLAIR AG: Opioid use in fibromyalgia: a cautionary tale. *Mayo Clin Proc* 2016; 91: 640-8.
29. ADLER-NEAL AL, ZEIDAN F: Mindfulness meditation for fibromyalgia: mechanistic and clinical considerations. *Curr Rheumatol Rep* 2017; 19: 59.
30. HOGAN KA, CCS C, CHINI EN: The multi-faceted ecto-enzyme CD38: roles in immunomodulation, cancer, aging, and metabolic diseases. *Front Immunol* 2019; 10: 1187.
31. FELDMAN R, MONAKHOV M, PRATT M, EBSTEIN RP: Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. *Biol Psychiatry* 2016; 79: 174-84.
32. NING L, SHAN G, SUN Z *et al*.: Quantitative proteomic analysis reveals the deregulation

of nicotinamide adenine dinucleotide metabolism and CD38 in inflammatory bowel disease. *Biomed Res Int* 2019; 2019: 3950628.
33. SADEGHI L, YEKTA R, DEHGHAN G: New mechanisms of phenytoin in calcium homeostasis: competitive inhibition of CD38 in hippocampal cells. *Daru* 2018; 26: 191-8.
34. HULL LC, RABENDER C, GABRA BH, ZHANG F, LI PL, DEWEY WL: Role of CD38, a cyclic ADP-ribosylcyclase, in morphine antinociception and tolerance. *J Pharmacol Exp Ther* 2010; 334: 1042-50.
35. ZHAO Y, HUANG G, WU Q *et al*.: Cryo-EM structures of apo and antagonist-bound human Cav3.1. *Nature* 2019; 576: 492-7.
36. ROBOON J, HATTORI T, ISHII H *et al*.: Deletion of CD38 suppresses glial activation and neuroinflammation in a mouse model of demyelination. *Front Cell Neurosci* 2019; 13: 258.
37. YU J, TANG YY, WANG RR *et al*.: A critical time window for the analgesic effect of central histamine in the partial sciatic ligation model of neuropathic pain. *J Neuroinflammation* 2016; 13: 163.
38. JIN EH, ZHANG E, KO Y *et al*.: Genome-wide expression profiling of complex regional pain syndrome. *PLoS One* 2013; 8: e79435.
39. MUNARI L, PROVENSI G, PASSANI MB *et al*.: Brain histamine is crucial for selective serotonin reuptake inhibitors' behavioral and neurochemical effects. *Int J Neuropsychopharmacol* 2015; 18: pyv045.
40. SUN Q, ZENG QC, CHEN YQ, ZHANG M, WEI LL, CHEN P: Long intergenic noncoding RNA p21 suppresses the apoptosis of hippocampus neurons in streptozotocin-diabetic mice by sponging microRNA-221 through upregulation of FOS. *J Cell Physiol* 2019; 234: 21113-25.
41. TOUJ S, TOKUNAGA R, AL AS, BRONCHTI G, PICHE M: Pain hypersensitivity is associated with increased amygdala volume and c-Fos immunoreactivity in anophthalmic mice. *Neuroscience* 2019; 418: 37-49.
42. BORYSOVYCH BV, BOGDANOVA OV, LOMBARD A *et al*.: Cortical spreading depression decreases Fos expression in rat periaqueductal gray matter. *Neurosci Lett* 2015; 585: 138-43.
43. NASCIMENTO SS, CAMARGO EA, DESANTANA JM *et al*.: Linalool and linalool complexed in beta-cyclodextrin produce anti-hyperalgesic activity and increase Fos protein expression in animal model for fibromyalgia. *Naunyn Schmiedebergs Arch Pharmacol* 2014; 387: 935-42.
44. SCHERNTHANER G, SCHERNTHANER GH: The right place for Metformin today. *Diabetes Res Clin Pract* 2019; 159: 107946.
45. SOUKAS AA, HAO H, WU L: Metformin as Anti-Aging Therapy: Is It for Everyone. *Trends Endocrinol Metab* 2019; 30: 745-55.
46. FORETZ M, GUIGAS B, VIOLLET B: Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat Rev Endocrinol* 2019; 15: 569-89.
47. NEUMANN B, BAROR R, ZHAO C *et al*.: Metformin restores CNS remyelination capacity by rejuvenating aged stem cells. *Cell Stem Cell* 2019; 25: 473-485.e8.
48. ALCOCER-GOMEZ E, GARRIDO-MARAVER J, BULLON P *et al*.: Metformin and caloric restriction induce an AMPK-dependent restoration of mitochondrial dysfunction in fibroblasts from Fibromyalgia patients. *Biochim Biophys Acta* 2015; 1852: 1257-67.
49. BULLON P, ALCOCER-GOMEZ E, CARRION AM *et al*.: AMPK phosphorylation modulates pain by activation of NLRP3 inflammasome. *Antioxid Redox Signal* 2016; 24: 157-70.