Original Article

Screening of differentially expressed genes in children with cerebral palsy and the construction of a network of the effective components of traditional Chinese medicine

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Background: The study sought to construct a network of the effective components of traditional Chinese medicine (TCM) and potential therapeutic target genes of cerebral palsy based on data sets from high-throughput sequencing and the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP).

Methods: A transcriptome sequencing data set (GSE183021) of blood samples from children with cerebral palsy was downloaded from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) between the cerebral palsy blood samples and control blood samples were screened. The TCM active components and target genes were identified from the TCMSP. We constructed a network of the active ingredients of TCM and the cerebral palsy DEGs.

Results: Using a |log2 fold change| ≥1 and a false discovery rate <0.05 as the screening criteria for the blood samples of 5 children with cerebral palsy and 5 control participants, 399 DEGs were identified. In the cerebral palsy blood samples, 209 genes were upregulated, and 190 genes were downregulated. The effective components of Angelica sinensis, Shenjincao, and Achyranthes bidentata, targeted 158 genes, and 49 genes crossed with the cerebral palsy DEGs. A network was constructed with the active ingredients of Angelica sinensis, Shenjincao, and Achyranthes bidentata and the DEGs of the cerebral palsy as nodes. *Interleukin* (*IL*)*-1β*, *IL-6*, *prostaglandin-endoperoxide synthase 1*, *tumor necrosis factor*, *estrogen receptor 1*, and *nitric oxide synthase 2* had a wide range of effects on the effective components of Angelica sinensis, Shenjincao, and Achyranthes bidentata.

Conclusions: The effective components of Angelica sinensis, Shenjincao, Achyranthes sinensis, and interact closely with the cerebral palsy DEGs. Based on the interaction network, the pharmacological mechanism of TCM in the treatment of cerebral palsy can be elucidated and new therapeutic targets discovered.

Keywords: Traditional Chinese medicine (TCM); cerebral palsy; network

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Introduction

Cerebral palsy is the most common disabling disease among children, and includes motor and postural development disorders (1). Motor dysfunction is the core symptom of cerebral palsy (1). An epidemiological study shown that the overall prevalence of cerebral palsy is between 15–35% (2). Preterm birth, maternal infection during pregnancy, low body weight, respiratory distress, and amniotic fluid contamination are high-risk factors for neonatal cerebral palsy (3). Cerebral palsy places enormous burdens (both mental and economic) on families and societies (4). The quality of life of children with cerebral palsy is generally low. To date, the pathogenesis of pediatric cerebral palsy has not yet been completely clarified. It is widely believed that cerebral palsy is related to the joint action of multiple factors in the perinatal period, and hypoxia may be the critical link (5).

Traditional Chinese medicine (TCM) has a good effect in treating infantile cerebral palsy. Angelica sinensis, Shenjincao, Achyranthes bidentata, and other drugs promote blood circulation and menstruation (6-8). However, pharmacological mechanism of TCM is not clear. The rise of network pharmacology and high-throughput omics data analysis technology have created conditions for explaining the occurrence and development of diseases from the perspective of system biology and biological network balance, understanding interactions between drugs and the body with the overall aim of improving or restoring biological network balance, and guiding the discovery of new medicines. Unfortunately, the research on the treatment of cerebral palsy with traditional Chinese medicine is limited to a certain drug or a certain target, and there is no macroscopic screening of key gene sets or construction of drug-gene interaction networks.

We screened differentially expressed gene sets in cerebral palsy blood samples based on high-throughput sequencing data. We obtained the active ingredients of TCM for the treatment of cerebral palsy and the gene set of active ingredients in the TCM pharmacology network database. We de-intersected the two gene sets and constructed an interaction network between TCM active ingredients and potential therapeutic target genes. This may provide clues for elucidating the pharmacological mechanism of TCM in the treatment of cerebral palsy and discovering potential therapeutic targets. We present the following article in accordance with the STREGA reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-171/rc).

Methods

Data download

In this study, a whole transcriptome sequencing data set (GSE183021) of the blood samples of children with cerebral palsy was downloaded from the Gene Expression Omnibus (GEO) database. The data set included blood samples from 5 children with cerebral palsy and 5 control subjects. Batch correction and normalization were performed on the data set, and the ribonucleic acid expression profile was summarized, logarithmically transformed, and combined into a matrix. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Differential gene screening

Fold change (FC) was calculated as follows: the gene expression of blood samples from patients with cerebral palsy/the gene expression of blood samples from control subjects. The differential genes were screened using the rank-sum test. The differentially expressed genes (DEGs) were screened using the following criteria: a $|\log2FC| > 1$, and a false discovery rate (FDR) <0.05.

Gene Ontology (GO) enrichment analysis

A GO functional enrichment analysis was carried out for the differential genes, using David data, and an FDR <0.05 was used as the screening condition. The results of the enrichment analysis were visualized using R package (ggplot).

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The differential genes were analyzed by the KEGG. The KEGG pathway enrichment analysis was carried out using KEGG data, and an FDR <0.05 was used as the screening condition. The results of the enrichment analysis were visualized using R package (ggplot).

Construction of effective components and target gene network of TCM

The effective components and target genes of TCM were identified from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). The target genes of TCM combined with the cerebral

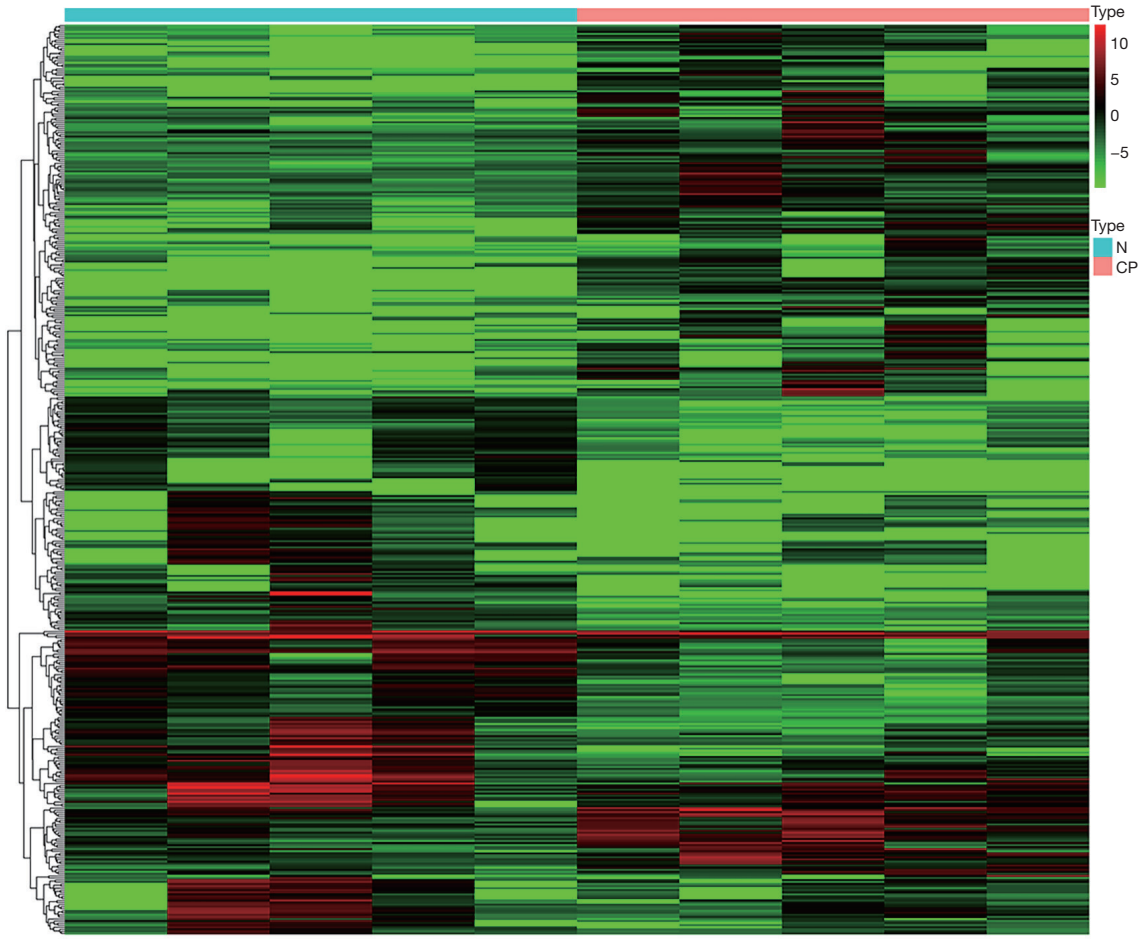

Figure 1 Five heat maps of DEGs in the blood samples of 5 children with cerebral palsy and 5 control subjects. N, normal blood samples; CP, cerebral palsy blood samples; DEGs, differentially expressed genes.

palsy DEGs from the blood samples were collected, and all the data were imported into Cytoscape 3.61. The effective components and a target gene network of TCM were constructed using the software.

Statistical analysis

All the statistical data in this study were analyzed by R (V3.5.1) and related R package. An FDR <0.05 was considered statistically significant.

Results

Screening of DEGs in cerebral palsy

Taking a | log2FC| ≥1 and an FDR <0.05 as the screening criteria, 399 DEGs were screened from the blood samples of 5 children with cerebral palsy and 5 control subjects. A total of 209 genes were downregulated in the blood samples of children with cerebral palsy, and 190 genes were upregulated. The heat map of the DEGs between the cerebral palsy and control subjects is shown in *Figure 1*. The top 20 genes with the smallest FDRs are shown in *Table 1*.

GO enrichment analysis of the DEGs in cerebral palsy

A GO enrichment analysis was performed on the top 20 DEGs in the cerebral palsy samples with the smallest FDRs. The cerebral palsy DEGs were mainly related to immune response, cell activation, and neurogenesis. In terms of the biological processes, the DEGs were primarily associated with the inherent components of axons, membrane regions, presynaptic membrane components,

Table 1 The first 20 DEGs with the smallest FDRs in the cerebral palsy samples

Gene	Cerebral palsy	Control	Log_2FC	FDR
CXCL8	3.23	0.03	6.67	0.001
UNC45B	2.16	0.02	–6.60	0.001
ARC	17.87	0.23	–6.28	0.001
TNF	23.41	0.37	5.99	0.001
TCEAL6	1.37	0.02	5.94	0.001
IL1B	45.14	3.08	–5.56	0.002
NOS2	3.02	0.10	7.96	0.002
PEBP4	1.62	0.06	–4.88	0.002
IL4	0.69	0.02	6.83	0.002
RSPO2	4.51	0.16	–4.78	0.002
BAX	1.57	0.06	4.74	0.003
VEGFD	6.91	0.28	–4.60	0.003
F7	0.56	0.02	4.49	0.003
SFRP5	6.51	0.29	–4.47	0.003
ADRB3	1.26	0.06	5.41	0.004
CMTM5	0.80	0.04	–6.38	0.004
CCL5	0.66	0.03	2.38	0.004
CDK5R2	0.87	0.05	–3.28	0.004
PCOLCE2	4.78	0.25	–4.24	0.004
MAPK8	0.42	0.02	–4.23	0.004

DEGs, differentially expressed genes; FDR, false discovery rate; FC, fold change.

cytoskeleton components, and cell connections. In terms of the cellular components, the DEGs were mainly related to antigen binding, catecholamine binding, antioxidant activity, and the cytoskeleton components. The results are shown in *Figure 2*.

KEGG enrichment analysis of DEGs in cerebral palsy

A KEGG enrichment analysis was performed on the top 20 cerebral palsy DEGs with the smallest FDRs. Taking an FDR <0.05 as the screening criteria, 11 significantly enriched pathways were ultimately identified. The signaling pathways involved in the immune system and inflammatory response included Th1 cell differentiation, the toll-like receptor signaling pathway, the *Nucleotide-binding and oligomerization domain* (*NOD*)-like receptor signaling pathway, *Th1* and *Th2* cell differentiation, natural-killer cell-mediated cytotoxicity, antigen processing and presentation, and cytokine receptor interaction. The signaling pathways involved in the nervous system included the *phosphatidylinositol 3-kinase* (*PI3K*)-*Akt* signal pathway, and the hypoxia-inducible factor-1 (*HIF-1*) signaling pathway. Other pathways included the cell cycle, and the cytoplasmic deoxyribonucleic acid (DNA) sensing pathway. The KEGG enrichment analysis showed that the cerebral palsy DEGs were mainly related to the immune and inflammatory response signaling pathways and the nervous system signaling pathways. The results are shown in *Figure 3*.

Prediction of effective components of TCM

A database analysis of the effective components of TCM revealed that 3 effective ingredients (i.e., Angelica sinensis, Shenjincao, and Achyranthes bidentata) were mainly used to treat infantile cerebral palsy. The results are shown in *Table 2*.

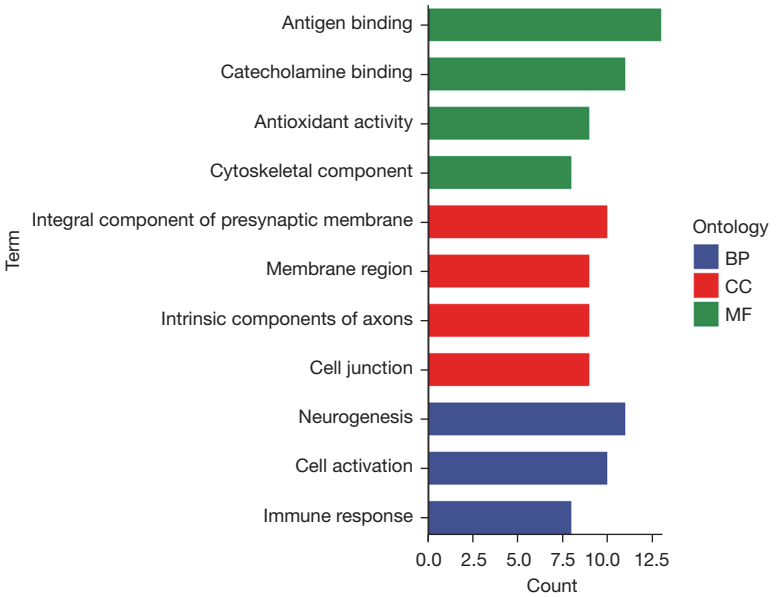

Figure 2 GO enrichment analysis of DEGs in children with cerebral palsy. BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology; DEGs, differentially expressed genes.

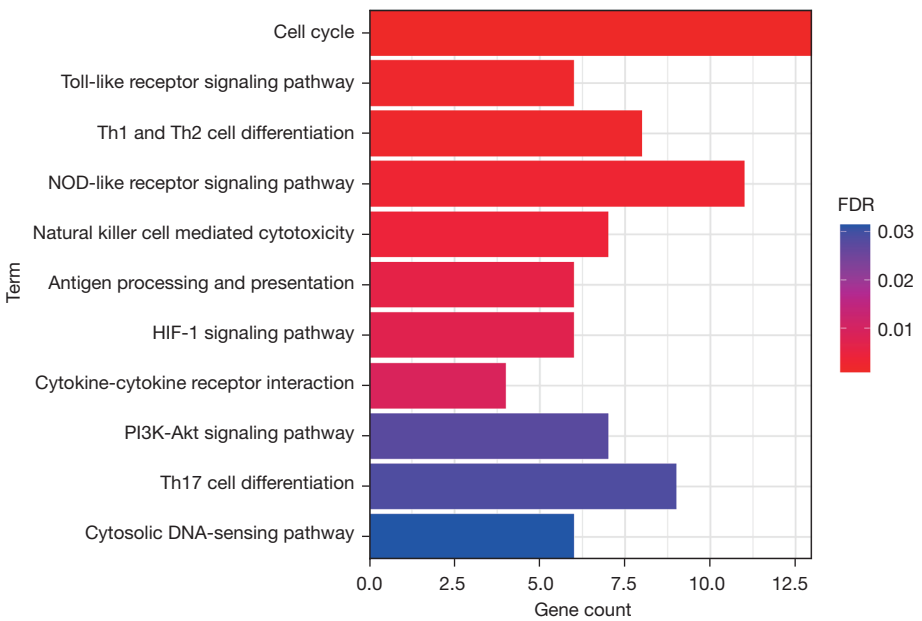

Figure 3 KEGG enrichment analysis of DEGs in children with cerebral palsy. FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

Intersection of target genes and the differential genes of TCM

The active components of Angelica sinensis, Shenjincao, and Achyranthes bidentata, targeted 158 genes, and 49 genes crossed with the cerebral palsy DEGs (see *Figure 4*).

TCM and construction of differential gene network

The active components of Angelica sinensis, Shenjincao, and Achyranthes bidentata, and the cerebral palsy DEGs were taken as the nodes to construct the action network

Table 2 Analysis of main components of Angelica sinensis, Shenjincao, and Achyranthes bidentata

Drug	ID	Name
Angelica sinensis	MOL004214	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one
	MOL003564	Mandenol
	MOL003541	Ethyl oleate (NF)
	MOL001833	Beta-sitosterol
	MOL002455	Stigmasterol
	MOL002186	Quercetin
	MOL000643	1-Monolinolein
	MOL000586	((1S)-3-((E)-but-2-enyl)-2-methyl-4-oxo-1-cyclopent
	MOL001674	Sexangularetin
	MOL004932	Beta-sitosterol
	MOL000936	Sitosterol
	MOL000421	Berberine
Shenjincao	MOL002471	Berberrubine
	MOL008611	Epiberberine
	MOL006531	(R)-Canadine
	MOL000231	Berlambine
	MOL000912	Corchoroside A_qt
Achyranthes bidentata	MOL001340	Magnograndiolide
	MOL000784	Palmatine
	MOL000936	Sitosterol
	MOL000421	Berberine
	MOL000912	Corchoroside A_qt

(see *Figure 5*). *Interleukin* (*IL*)*-1B*, *IL-6*, *prostaglandin-endoperoxide synthase 1* (*PTGS1*), *tumor necrosis factor* (*TNF*), *estrogen receptor 1* (*ESR1*), *nitric oxide synthase 2* (*NOS2*), and other genes played an important role with the effective components of TCM.

Discussion

This study analyzed a transcriptome sequencing data set of blood samples of children with cerebral palsy, screened the DEGs in the blood samples of the children with cerebral palsy and control subjects. We have 190 up-regulated genes and 209 down-regulated genes. We believe that there are more down-regulated genes than up-regulated genes, and the sample size may be related to our screening criteria. We conducted an enrichment analysis of those genes. The GO and KEGG enrichment analyses showed that these DEGs were related to the immune response, neurogenesis, inherent components of axons, presynaptic membrane components, cell connection, antioxidant activity, immune response, and nervous system signaling pathways. Our results provide insights into the pathogenesis of DEGs, and confirmed the representativeness of these DEGs, which may play key roles in the pathogenesis of cerebral palsy.

Consistent with our results, previous studies (4,5) have shown that cerebral palsy may be related to natural-killer cell-mediated cytotoxicity, and antigen processing and presentation. This study obtained the relevant information about the TCM components and targets, such as Angelica sinensis, Shenjincao, and Achyranthes bidentata, from the TCMSP. An interaction network was then constructed by combining the DEGs. *IL1B*, *IL6*, *PTGS1*, *TNF*, toll-like

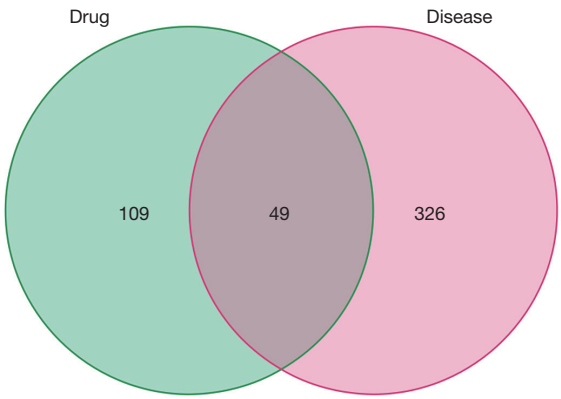

Figure 4 Intersection of target genes of the active components of Angelica sinensis, Shenjincao and Achyranthes bidentata and DEGs in cerebral palsy. DEGs, differentially expressed genes.

receptor 4 (*TLR4*), *progesterone receptor* (*PGR*), *nitric oxide synthase* (*NOS2*), and other genes were shown to play an important role in the effective TCM components. We also found that these target genes were closely related to nervous system development and diseases.

Additionally, we found that *IL-6*, *IL-1B*, and TNF are the best protein biomarkers for predicting neurodevelopment injury (9). *IL-6* is an important neuroinflammatory factor that mediates brain inflammatory lesions and amyloidosis, and is associated with a variety of brain diseases, including Alzheimer's disease, and autism (10,11). *IL-6* is associated with periventricular leukomalacia and may affect neurodevelopment and increase the risk of cerebral palsy (12). Studies have shown that in children with cerebral palsy, cytokines are dysregulated, and plasma *TNF* levels

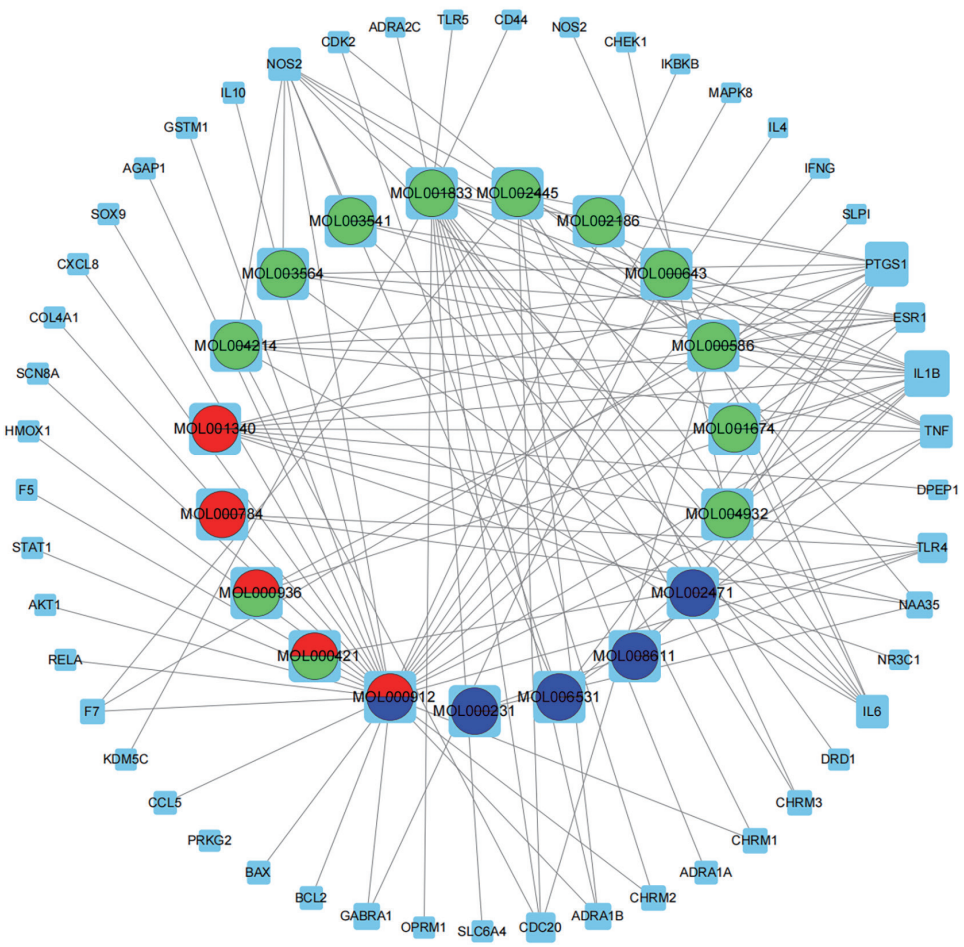

Figure 5 The active components of Angelica sinensis, Shenjincao, and Achyranthes bidentata, and the cerebral palsy DEGs were used as nodes to construct the action network. DEGs, differentially expressed genes. Red indicates Achyranthes bidentata, green indicates Angelica sinensis, dark blue indicates Shenjincao, and light blue indicates differential genes

are elevated (13,14). *IL-1B* plays a key role in inflammatory demyelinating polyradiculoneuropathy (15).

A Study also confirmed that the amplification of microsatellites in the *IL-1B* promoter and the *NOS2A* promoter may make children more prone to cerebral palsy after hypoxic-ischemic encephalopathy (16). *TLR4* belongs to the type I transmembrane protein, which is activated through both *MyD88* dependent and non-MyD88 dependent pathways, which activate macrophages and endothelial cells to promote the expression of inflammatory and immunomodulatory factor genes. A clinical study shown that *TLR4* polymorphism has a potential neuroprotective effect, which may be related to reducing the risk of cerebral palsy (17).

PTGS1 is considered a target for relieving pain and treating inflammation. In the neuroinflammatory response, the expression of *PTGS1* in astrocytes and microglia is significantly increased (18). *PTGS1* may be a pathological factor leading to neurodegeneration, mental disease, and epilepsy (18-20).

NOS2 promotes leukocyte migration to inflammatory sites via the endothelium and destroys the blood-brain barrier. The upregulation of *NOS2* occurs in various diseases, including autoimmune diseases, cancer, cardiovascular diseases, nervous system diseases, and inflammatory diseases (21-23). Under inflammatory conditions, elevated *NOS2* increases the risk of white-matter damage and cerebral palsy in children.

By combining GEO and TCMSP data, we constructed an action network with the active ingredients of Angelica sinensis, Shenjincao, Achyranthes bidentata and cerebral palsy differentially expressed genes as nodes, and screened out *IL1B*, *IL6*, *PTGS1*, *TNF*, *TLR4*, *PGR*, *NOS2* and other genes. These genes are all potential therapeutic targets. We believe that functional studies can be carried out based on these genes to verify the interaction between the effective components of TCM and genes, so as to determine therapeutic targets and innovate new drugs.

There are some flaws in this study. The first is the small amount of cerebral palsy blood samples used to analyze differential genes in this study, which needs to be further expanded with large sample size for verification. Second, this study lacks experimental validation *in vivo* and *in vitro* to support the results of our analysis. Finally, functional experiments of key genes need to be carried out to clarify the pathogenic mechanism and the mechanism of drug action. In sum, this study used a transcriptome sequencing data set to screen the DEGs of cerebral palsy samples. We constructed an action network with the active components of Angelica sinensis, Shenjincao, Achyranthes bidentata, and cerebral palsy DEGs as nodes by combining with the pharmacological network database of TCM. Our findings can be used to further clarify the pharmacological mechanism of TCM in the treatment of cerebral palsy and identify novel therapeutic targets.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-171/rc

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-171/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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