Original Article

Network pharmacology analysis of the mechanism of our hospital's experiential prescription in the treatment of Guillain Barré syndrome

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Background: This study aimed to explore the active ingredients and potential mechanism of our hospital's Guillain-Barré syndrome (GBS) experiential prescription in the treatment of GBS based on network pharmacology.

Methods: The traditional Chinese medicine system pharmacology (TCMSP) database was used to screen the active ingredients of the eight traditional Chinese medicines (TCMs) of the GBS-experiential prescription, and the Online Mendelian Inheritance in Man (OMIM), GeneCards, and MalaCards databases were used to obtain GBS-related gene targets. The common targets of the experiential prescriptions and GBS-related gene targets were acquired and imported into the STRING database to obtain the protein interaction relationship. Gene oncology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to predict the major mechanism of this prescription.

Results: The formula contained at least 154 potential active ingredients and a total of 4,270 unique targets, among which a total of 158 GBS-related disease targets and 70 common targets were found. The key targets included EGFR (Epidermal Growth Factor Receptor), TNF (Tumor Necrosis Factor), ITGAL (Integrin Subunit Alpha L), and CEBPA (CCAAT/Enhancer-Binding Protein Alpha), CPT2 (Carnitine Palmitoyltransferase 2), CRP (C-reactive protein), ICAM1 (Intercellular Adhesion Molecule 1), IL6 (interleukin 6), and PECAM1 (Platelet and Endothelial Cell Adhesion Molecule 1), CREBBP (CREB Binding Protein), etc. The GO enrichment analysis results revealed 116 terms, and the KEGG signaling pathway enrichment analysis results yielded 61 pathways, including influenza A, hepatitis B, malaria, etc.

Conclusions: The development of GBS and the mechanism underlying the effects of the GBS-experiential prescription have common and complex targets, which are worthy of in-depth exploration.

Keywords: Network pharmacology; Guillain-Barré syndrome (GBS); inflammatory demyelination; traditional Chinese medicine (TCM); disease target

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Introduction

Guillain-Barré syndrome (GBS) is an immune-related peripheral neuroinflammatory disease (1-3). As the pathogenesis of GBS has not yet been fully clarified, the effects of GBS treatment are limited. Typically, modern medicine uses symptomatic treatment and supportive strategies (e.g., neurotrophic therapy), and the overall outcome is not satisfactory. In order to enhance the effect of GBS treatment, our department developed a hospital-specific experiential traditional Chinese medicine (TCM)

prescription for the treatment of GBS. The composition of this prescription is as follows: astragalus 60 g, raw licorice 12 g, atractylodes 10 g, cork 10 g, jobstears seed 10 g, salvia 10 g, red peony 10 g, and Poria cocos 12 g. We previously applied this hospital GBS-experiential prescription (GBS-EP) for 10 years and found that it (alone or in combination with other treatments) can effectively benefit the treatment of GBS. In the follow-up observation, we noticed that the application of GBS-EP can improve the daily living ability score, Hughes limb function score, and help cure sensory dysfunction in patients.

Clinically, the manifestations of GBS are similar to "failure disease" and "heat evil" in TCM theory. The main TCM syndrome differentiation characteristics include Qi-and-Blood deficiency and pathogenic heat. Accordingly, based on TCM theory, the treatment strategy should be strengthening Qi, clearing damp-heat, cooling, and activating blood. However, the main active ingredients, molecular targets/mechanisms of GBS-EP remain largely unclear. In recent years, increasing studies have established the disease/gene-target/drug interaction network based on the analysis of network pharmacology to explore the therapeutic mechanism of TCM prescriptions (4-10). We conducted the present study using network pharmacology to determine the mechanism underlying the effects of GBS-EP at the molecular level, and our findings may provide references for discovering the etiology of GBS and further developing GBS treatment. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1743).

Methods

The key active ingredients and targets

The traditional Chinese medicine system pharmacology database and analysis platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) was employed, and the following TCM herbs were used to search for active ingredients: astragalus, raw licorice, atractylodes, cork, jobstears seed, salvia, red peony, and Poria cocos. The screening criteria for key active ingredients were set as follows: oral bioavailability (OB) ≥40%; and drug similarity (DL) ≥0.2. The corresponding gene targets for all key active ingredients were then acquired through the CTD database (http://ctdbase.org), and only the Homo sapiens target data were selected.

GBS associated targets

The Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org), Genecards (http://www.genecards.org), and Malacards (https://www.malacards.org) databases were used to search for GBS-associated targets. In the OMIM and Genecards databases, "Guillain-Barre syndrome" was used as the keyword, and in Malacards, the classic GBS subtype "acute inflammatory demyelinating polyneuropathy" was used as the keyword to obtain the targets.

Screening of common targets between GBS-EP and GBS

The targets of GBS-EP and the GBS-associated targets were listed respectively, and their intersection was drawn using a Venn diagram.

Protein-protein interaction (PPI) network

The above common targets were imported into the STRING database (http://string-db.org/cgi/input.pl), and the protein type was set as Homo sapiens. The interaction threshold value was set at 0.7, and only experimentally verified types of PPI were selected. The lonely island nodes were not displayed. The PPI network was generated, and the core targets were selected and paid special attention.

Enrichment analysis

Based on the common targets, the R package clusterProfiler was used to perform Gene oncology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Here, Gene Ratio was used to indicate the proportion of genes enriched to a specific GO or KEGG term in all targets, and multiple hypothesis tests were performed to adjust the P value calculation. The enrichment results were presented as bubble figures and detailed tables.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Statistical analyses were performed using R or the corresponding online tools as mentioned above. In

Table 1 The key ingredients of herbs in the GBS-EP

Herb	Number of key ingredients
Astragalus	12
Raw licorice	65
Atractylodes	3
Cork	15
Jobstears seed	2
Salvia	43
Red peony	17
Poria cocos	3

GBS-EP, GBS experiential formula.

enrichment analysis, multiple hypothesis tests were performed to adjust the P value calculation. The P values <0.05 were considered statistically significant.

Results

The active ingredients and corresponding targets of GBS-EP

Using the TCMSP database, we screened the active ingredients of astragalus, licorice, atractylodes, phellodendron, coix seed, salvia, red peony, and poria. A total of 160 key active ingredients were found under the criteria of OB ≥40% and DL ≥0.2; six repeated active ingredients were shared by multiple herbs and removed, and a total of 154 active ingredients were acquired. The specific key ingredients of each medicinal flavor are presented in *Table 1*. Next, using these 154 key active ingredients, a total of 8,102 targets were obtained via CTD database analysis. After de-duplication, 4,270 non-repetitive targets were obtained.

GBS associated targets

Using "Guillain-Barre Syndrome" as a keyword, we searched the OMIM and GeneCards databases, respectively. There were eight targets in the Genecards database and six targets in the OMIM database. Also, using the most classic GBS subtype, "acute inflammatory demyelinating polyneuropathy" as the keyword, 69 targets were found on the GeneCards database, and 109 targets were found on the OMIM database. Meanwhile, using Guillain-Barre Syndrome entry (MCID: GLL022) in the MalaCards database, 24 targets were found. After removing duplicates, there were a total of 158 targets.

Common targets

Among the 4,270 unique GBS-EP targets and 158 GBS associated targets, 70 common drug/disease targets were identified, and a Venn diagram was drawn to show the intersection (*Figure 1A*).

PPI network analysis

Using the STRING database, the PPI network of the above 70 common targets was established (*Figure 1B*), with 21 nodes and 15 edges. Among them, the target proteins EGFR (Epidermal Growth Factor Receptor), TNF (Tumor Necrosis Factor), ITGAL (Integrin Subunit Alpha L), and CREBBP (CREB Binding Protein) were in the core nodes of the PPI network, which may be highly significant for clarifying the treatment mechanism of GBS-EP.

Pathway enrichment analysis results

Furthermore, the 70 common targets were analyzed by GO and KEGG pathway enrichment, and the results are as follows. In the GO enrichment, a total of 116 enriched terms were found. As shown in *Table 2*, the enrichment of terms such as inflammatory response, extracellular space, positive regulation of gene expression, extrinsic apoptotic signaling pathway, and positive regulation of transcription from RNA polymerase II promoter may be the crucial mechanism of the therapeutic effects of GBS-EP, and the top 20 terms were presented using the bubble chart in *Figure 1C*. Meanwhile, a total of 61 KEGG pathways were enriched in the 70 common targets (*Table 3*). The top 20 pathways are shown in the bubble chart (*Figure 1D*), and the key KEGG pathways included influenza A, hepatitis B, malaria, etc.

Discussion

At present, there is no specific drug for GBS, and established treatment is generally based on immune-modulating treatment with plasma exchange or intravenous immunoglobulin in combination with supportive care (11-13). Recently, some novel drugs have been reported to be potentially effective, such as memantine, bifidobacterium, gabapentin, etc. (14-16). However, the effect of TCM was

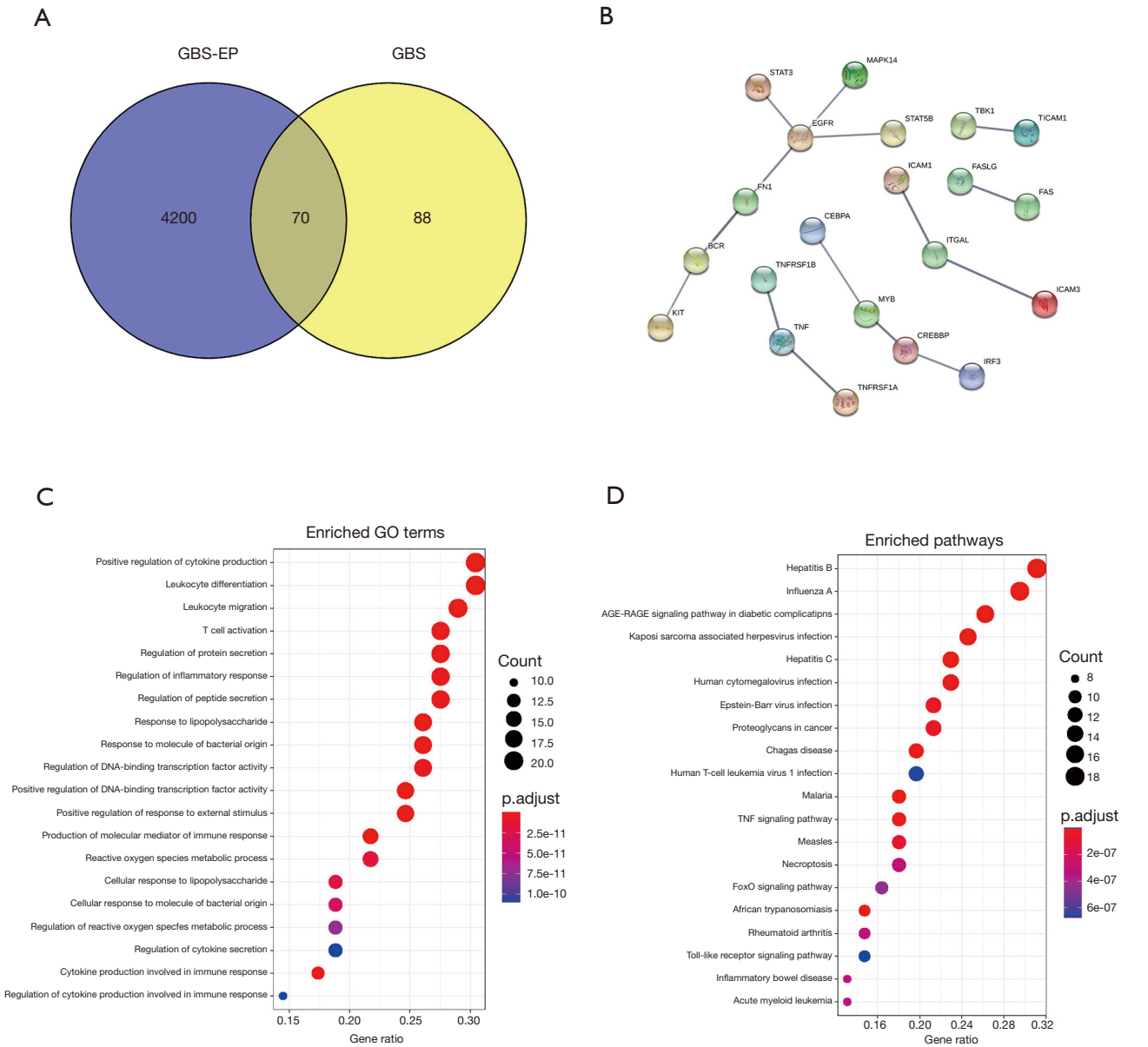

Figure 1 The key bioinformatics results of the GBS-EP and GBS-associated targets. (A) Venn diagram of the GBS-EP targets and GBS-associated targets; (B) the PPI networks of the common targets; (C) the bubble chart of the top 20 enriched GO terms based on the 70 common targets; (D) the bubble chart of the top 20 enriched KEGG terms based on the 70 common targets. GBS-EP, GBS experiential formula; PPI, protein-protein interaction; GO, Gene Oncology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

seldom investigated in GBS treatment. The application of network pharmacology in Chinese medicine has attracted the attention of researchers in recent years. Network pharmacology is a novel, promising, and cost-effective approach in discovering bioactive ingredients, predicting drug action targets, and analyzing drug action mechanisms from the perspective of biological network balance (17). It has been used in different diseases, such as ankylosing spondylitis, cancer, myocardial infarction, anemia, ulcerative colitis, etc. (17-21). The GBS-EP used in our hospital is composed of eight TCM herbs. After screening, a total of 154 active ingredients were obtained, such as the main active ingredients of astragalus syringae (including nicotinic acid, linolenic acid, proline, etc.), the main

Table 2 GO enrichment of the common targets

Category	Term	Count	%	Fold	FDR
BP	GO:0006954~inflammatory response	20	28.57	12.66	6.55E-13
CC	GO:0005615~extracellular space	29	41.43	5.61	2.15E-12
BP	GO:0010628~positive regulation of gene expression	16	22.86	14.65	8.92E-11
BP	GO:0097191~extrinsic apoptotic signaling pathway	9	12.86	51.40	2.37E-09
BP	GO:0045944~positive regulation of transcription from RNA polymerase II promoter	22	31.43	5.38	5.89E-08
CC	GO:0045121~membrane raft	11	15.71	13.90	3.69E-07
CC	GO:0009897~external side of plasma membrane	11	15.71	13.44	3.69E-07
BP	GO:0031663~lipopolysaccharide-mediated signaling pathway	7	10.00	52.48	6.72E-07
BP	GO:0051092~positive regulation of NF-kappaB transcription factor activity	10	14.29	18.04	6.72E-07
BP	GO:0043066~negative regulation of apoptotic process	15	21.43	7.91	6.72E-07
BP	GO:0006955~immune response	14	20.00	7.98	2.19E-06
BP	GO:0071222~cellular response to lipopolysaccharide	9	12.86	19.11	2.55E-06
BP	GO:0045429~positive regulation of nitric oxide biosynthetic process	7	10.00	39.05	2.55E-06
BP	GO:0032496~response to lipopolysaccharide	10	14.29	14.63	2.55E-06
CC	GO:0009986~cell surface	14	20.00	6.72	4.00E-06
BP	GO:0006919~activation of cysteine-type endopeptidase activity involved in apoptotic process	8	11.43	23.12	4.93E-06
BP	GO:0008285~negative regulation of cell proliferation	13	18.57	7.88	6.37E-06
BP	GO:0007165~signal transduction	20	28.57	4.13	1.17E-05
BP	GO:0043406~positive regulation of MAP kinase activity	7	10.00	28.46	1.23E-05
BP	GO:0097296~activation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway	5	7.14	92.26	1.43E-05
MF	GO:0005515~protein binding	58	82.86	1.59	2.59E-05
BP	GO:0050900~leukocyte migration	8	11.43	15.73	4.95E-05
MF	GO:0005125~cytokine activity	9	12.86	12.33	6.98E-05
BP	GO:0019221~cytokine-mediated signaling pathway	8	11.43	14.65	7.21E-05
BP	GO:0032755~positive regulation of interleukin-6 production	6	8.57	31.98	7.21E-05
CC	GO:0005576~extracellular region	20	28.57	3.23	1.65E-04
BP	GO:0008284~positive regulation of cell proliferation	12	17.14	6.18	1.80E-04
BP	GO:0007159~leukocyte cell-cell adhesion	5	7.14	47.98	1.86E-04
BP	GO:0045930~negative regulation of mitotic cell cycle	5	7.14	46.13	2.09E-04
BP	GO:0007568~aging	8	11.43	11.63	2.64E-04
BP	GO:0051607~defense response to virus	8	11.43	11.63	2.64E-04

Table 2 (*continued*)

Table 2 (*continued*)

Category	Term	Count	%	Fold	FDR
BP	GO:0070374~positive regulation of ERK1 and ERK2 cascade	8	11.43	10.97	3.74E-04
BP	GO:0033209~tumor necrosis factor-mediated signaling pathway	7	10.00	14.23	4.29E-04
BP	GO:0050729~positive regulation of inflammatory response	6	8.57	19.72	5.71E-04
BP	GO:0030198~extracellular matrix organization	8	11.43	9.79	6.98E-04
MF	GO:0042802~identical protein binding	14	20.00	4.51	7.24E-04
BP	GO:0008625~extrinsic apoptotic signaling pathway via death domain receptors	5	7.14	31.56	7.28E-04
BP	GO:0042517~positive regulation of tyrosine phosphorylation of Stat3 protein	5	7.14	31.56	7.28E-04
BP	GO:0042060~wound healing	6	8.57	17.99	7.59E-04
BP	GO:0006953~acute-phase response	5	7.14	30.75	7.59E-04
BP	GO:0051897~positive regulation of protein kinase B signaling	6	8.57	17.13	9.27E-04
BP	GO:1904707~positive regulation of vascular smooth muscle cell proliferation	4	5.71	63.97	0.001081
CC	GO:0031093~platelet alpha granule lumen	5	7.14	23.67	0.001424
BP	GO:0008630~intrinsic apoptotic signaling pathway in response to DNA damage	5	7.14	25.52	0.001467
BP	GO:0045893~positive regulation of transcription, DNA-templated	11	15.71	5.12	0.001581
BP	GO:0043123~positive regulation of I-kappaB kinase/NF-kappaB signaling	7	10.00	10.43	0.001754
BP	GO:0043536~positive regulation of blood vessel endothelial cell migration	4	5.71	50.50	0.002035
CC	GO:0005886~plasma membrane	31	44.29	1.96	0.002038
BP	GO:0002576~platelet degranulation	6	8.57	13.97	0.002094
BP	GO:0051091~positive regulation of sequence-specific DNA binding transcription factor activity	6	8.57	13.71	0.002213
BP	GO:1902895~positive regulation of pri-miRNA transcription from RNA polymerase II promoter	4	5.71	47.98	0.002213
BP	GO:0001666~response to hypoxia	7	10.00	9.76	0.002231
BP	GO:0050776~regulation of immune response	7	10.00	9.43	0.002634
BP	GO:0031334~positive regulation of protein complex assembly	4	5.71	43.62	0.0027
BP	GO:0000165~MAPK cascade	8	11.43	7.32	0.0027
BP	GO:0071407~cellular response to organic cyclic compound	5	7.14	20.33	0.0027
BP	GO:0006915~apoptotic process	11	15.71	4.65	0.0027
BP	GO:0030335~positive regulation of cell migration	7	10.00	9.13	0.002832
BP	GO:0042127~regulation of cell proliferation	7	10.00	9.08	0.002858

Table 2 (*continued*)

Table 2 (*continued*)

Category	Term	Count	%	Fold	FDR
MF	GO:0008201~heparin binding	7	10.00	10.55	0.002912
CC	GO:0070062~extracellular exosome	24	34.29	2.22	0.003261
BP	GO:0050715~positive regulation of cytokine secretion	4	5.71	38.38	0.003546
BP	GO:0060326~cell chemotaxis	5	7.14	18.45	0.003546
BP	GO:0051384~response to glucocorticoid	5	7.14	18.45	0.003546
BP	GO:0001934~positive regulation of protein phosphorylation	6	8.57	11.33	0.004148
BP	GO:0032728~positive regulation of interferon-beta production	4	5.71	35.54	0.004223
BP	GO:0033138~positive regulation of peptidyl-serine phosphorylation	5	7.14	17.13	0.004471
BP	GO:0097190~apoptotic signaling pathway	5	7.14	16.89	0.004557
BP	GO:0060333~interferon-gamma-mediated signaling pathway	5	7.14	16.89	0.004557
CC	GO:0072562~blood microparticle	6	8.57	10.28	0.004729
BP	GO:0043065~positive regulation of apoptotic process	8	11.43	6.40	0.004922
BP	GO:0042981~regulation of apoptotic process	7	10.00	7.88	0.005101
BP	GO:2000553~positive regulation of T-helper 2 cell cytokine production	3	4.29	119.94	0.005101
BP	GO:0097527~necroptotic signaling pathway	3	4.29	119.94	0.005101
BP	GO:0008360~regulation of cell shape	6	8.57	10.28	0.005581
BP	GO:0050728~negative regulation of inflammatory response	5	7.14	15.18	0.006221
BP	GO:0050731~positive regulation of peptidyl-tyrosine phosphorylation	5	7.14	14.63	0.007063
BP	GO:0050830~defense response to Gram-positive bacterium	5	7.14	14.11	0.007892
BP	GO:0018108~peptidyl-tyrosine phosphorylation	6	8.57	9.41	0.007892
BP	GO:0006928~movement of cell or subcellular component	5	7.14	13.95	0.00809
MF	GO:0042803~protein homodimerization activity	12	17.14	3.96	0.008157
BP	GO:0035234~ectopic germ cell programmed cell death	3	4.29	89.96	0.008374
BP	GO:0010888~negative regulation of lipid storage	3	4.29	89.96	0.008374
MF	GO:0004713~protein tyrosine kinase activity	6	8.57	10.88	0.008484
BP	GO:0032930~positive regulation of superoxide anion generation	3	4.29	79.96	0.010584
BP	GO:0001541~ovarian follicle development	4	5.71	22.85	0.011894
BP	GO:0045599~negative regulation of fat cell differentiation	4	5.71	22.85	0.011894
BP	GO:0032760~positive regulation of tumor necrosis factor production	4	5.71	20.42	0.016107
BP	GO:0051781~positive regulation of cell division	4	5.71	20.42	0.016107
CC	GO:0016020~membrane	19	27.14	2.25	0.016874
BP	GO:0032727~positive regulation of interferon-alpha production	3	4.29	59.97	0.017967

Table 2 (*continued*)

Table 2 (*continued*)

Category	Term	Count	%	Fold	FDR
BP	GO:0071356~cellular response to tumor necrosis factor	5	7.14	10.90	0.01801
BP	GO:0007157~heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	4	5.71	19.19	0.018547
BP	GO:0050901~leukocyte tethering or rolling	3	4.29	55.36	0.020363
MF	GO:0046982~protein heterodimerization activity	9	12.86	4.67	0.020606
BP	GO:0048146~positive regulation of fibroblast proliferation	4	5.71	17.77	0.022342
BP	GO:0016032~viral process	7	10.00	5.62	0.022342
BP	GO:0006935~chemotaxis	5	7.14	9.83	0.024779
BP	GO:0008631~intrinsic apoptotic signaling pathway in response to oxidative stress	3	4.29	47.98	0.025624
BP	GO:0060397~JAK-STAT cascade involved in growth hormone signaling pathway	3	4.29	47.98	0.025624
CC	GO:0005829~cytosol	24	34.29	1.88	0.025833
MF	GO:0005178~integrin binding	5	7.14	11.48	0.026985
BP	GO:0007249~I-kappaB kinase/NF-kappaB signaling	4	5.71	15.99	0.027547
BP	GO:0050679~positive regulation of epithelial cell proliferation	4	5.71	15.99	0.027547
BP	GO:0042102~positive regulation of T cell proliferation	4	5.71	15.99	0.027547
BP	GO:0048661~positive regulation of smooth muscle cell proliferation	4	5.71	15.99	0.027547
BP	GO:0044130~negative regulation of growth of symbiont in host	3	4.29	44.98	0.027547
BP	GO:0032722~positive regulation of chemokine production	3	4.29	42.33	0.030446
BP	GO:0042523~positive regulation of tyrosine phosphorylation of Stat5 protein	3	4.29	42.33	0.030446
BP	GO:0010629~negative regulation of gene expression	5	7.14	8.75	0.033418
BP	GO:0030225~macrophage differentiation	3	4.29	39.98	0.033418
BP	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	10	14.29	3.33	0.035781
BP	GO:0007155~cell adhesion	8	11.43	4.18	0.036558
BP	GO:0071260~cellular response to mechanical stimulus	4	5.71	13.51	0.041224
BP	GO:0046427~positive regulation of JAK-STAT cascade	3	4.29	32.71	0.047843
BP	GO:0030324~lung development	4	5.71	12.63	0.04894

GO, gene oncology; BP, biological process; CC, cellular component; MF, molecular function; FDR, false discovery rate.

active ingredients of licorice (including glycol, good rotol, isorhamnetin, cadherin, naringin, glycyrrhizin, etc.), the main active ingredients of atractylodes (including 3-acetoxy atractylone, 2-hydroxyisooxypropyl-3-hydroxy-7-iso Pentene-2,3-dihydrobenzofuran-5-carboxylic acid, etc.), the main active ingredients of cork (including opazone, pterin, dehydrotanshinone II A, niromycin, rutin, quercetin, etc.), the main active ingredients of coix seed (e.g., sitosterol), the main active ingredients of salvia (including paclitaxel, isoperatorin, dehydrotanshinone II A, gallate, formyl

Table 3 KEGG pathway enrichment of the common targets

Term	Count	%	Fold	FDR
hsa05164:Influenza A	18	25.71	11.86	3.55E-12
hsa05161:Hepatitis B	16	22.86	12.65	3.10E-11
hsa05144:Malaria	11	15.71	25.74	2.03E-10
hsa05200:Pathways in cancer	20	28.57	5.83	6.52E-09
hsa05142:Chagas disease (American trypanosomiasis)	12	17.14	13.23	1.67E-08
hsa05143:African trypanosomiasis	8	11.43	27.79	1.40E-07
hsa04668:TNF signaling pathway	11	15.71	11.79	2.55E-07
hsa05168:Herpes simplex infection	13	18.57	8.14	4.08E-07
hsa05205:Proteoglycans in cancer	13	18.57	7.45	9.74E-07
hsa05162:Measles	11	15.71	9.48	1.39E-06
hsa05166:HTLV-I infection	14	20.00	6.32	1.39E-06
hsa05323:Rheumatoid arthritis	9	12.86	11.73	5.59E-06
hsa04060:Cytokine-cytokine receptor interaction	13	18.57	6.13	5.61E-06
hsa05321:Inflammatory bowel disease (IBD)	8	11.43	14.33	7.12E-06
hsa05160:Hepatitis C	10	14.29	8.62	1.05E-05
hsa04068:FoxO signaling pathway	10	14.29	8.56	1.05E-05
hsa05152:Tuberculosis	11	15.71	7.13	1.21E-05
hsa04620:Toll-like receptor signaling pathway	9	12.86	9.73	1.55E-05
hsa05133:Pertussis	8	11.43	12.23	1.56E-05
hsa05145:Toxoplasmosis	9	12.86	9.38	1.85E-05
hsa05221:Acute myeloid leukemia	7	10.00	14.33	3.37E-05
hsa04514:Cell adhesion molecules (CAMs)	9	12.86	7.27	1.11E-04
hsa05140:Leishmaniasis	7	10.00	11.30	1.23E-04
hsa04932:Non-alcoholic fatty liver disease (NAFLD)	9	12.86	6.83	1.59E-04
hsa04064:NF-kappa B signaling pathway	7	10.00	9.22	3.59E-04
hsa05332:Graft-versus-host disease	5	7.14	17.37	6.32E-04
hsa05212:Pancreatic cancer	6	8.57	10.58	7.98E-04
hsa05330:Allograft rejection	5	7.14	15.49	9.15E-04
hsa04010:MAPK signaling pathway	10	14.29	4.53	9.15E-04
hsa04622:RIG-I-like receptor signaling pathway	6	8.57	9.83	9.86E-04
hsa04920:Adipocytokine signaling pathway	6	8.57	9.83	9.86E-04
hsa05169:Epstein-Barr virus infection	7	10.00	6.58	0.001779502
hsa04151:PI3K-Akt signaling pathway	11	15.71	3.66	0.001844849
hsa05014:Amyotrophic lateral sclerosis (ALS)	5	7.14	11.46	0.002439226
hsa04210:Apoptosis	5	7.14	9.25	0.005317397

Table 3 (*continued*)

Table 3 (*continued*)

Term	Count	%	Fold	FDR
hsa05146:Amoebiasis	6	8.57	6.49	0.005621139
hsa04623:Cytosolic DNA-sensing pathway	5	7.14	8.96	0.005656646
hsa04670:Leukocyte transendothelial migration	6	8.57	5.98	0.007605907
hsa04917:Prolactin signaling pathway	5	7.14	8.07	0.007849669
hsa04650:Natural killer cell mediated cytotoxicity	6	8.57	5.64	0.009324623
hsa04062:Chemokine signaling pathway	7	10.00	4.31	0.011938051
hsa05219:Bladder cancer	4	5.71	11.19	0.011949943
hsa04940:Type I diabetes mellitus	4	5.71	10.92	0.012494005
hsa04640:Hematopoietic cell lineage	5	7.14	6.59	0.014401449
hsa04550:Signaling pathways regulating pluripotency of stem cells	6	8.57	4.91	0.014832723
hsa04672:Intestinal immune network for IgA production	4	5.71	9.76	0.016004866
hsa04630:Jak-STAT signaling pathway	6	8.57	4.74	0.016251128
hsa05203:Viral carcinogenesis	7	10.00	3.91	0.016251128
hsa04015:Rap1 signaling pathway	7	10.00	3.82	0.017832046
hsa04066:HIF-1 signaling pathway	5	7.14	5.97	0.017867158
hsa05320:Autoimmune thyroid disease	4	5.71	8.82	0.019089807
hsa04660:T cell receptor signaling pathway	5	7.14	5.73	0.019773142
hsa04621:NOD-like receptor signaling pathway	4	5.71	8.19	0.022491681
hsa04014:Ras signaling pathway	7	10.00	3.55	0.022744545
hsa05202:Transcriptional misregulation in cancer	6	8.57	4.12	0.024897494
hsa05210:Colorectal cancer	4	5.71	7.40	0.028012543
hsa05211:Renal cell carcinoma	4	5.71	6.95	0.032508076
hsa04071:Sphingolipid signaling pathway	5	7.14	4.78	0.032740155
hsa05220:Chronic myeloid leukemia	4	5.71	6.37	0.039485241
hsa04380:Osteoclast differentiation	5	7.14	4.38	0.042128115
hsa05310:Asthma	3	4.29	11.47	0.043777439

KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

tanshinone, etc.), the main active ingredients of red peony (including ellagic acid, paeoniflorin, spinach sterol, 9-ethyl-neo-cocoside A, etc.), and the main active ingredients of poria cocos (including ergosterol-3β-ol, ergosterol peroxide, and dehydroepidermis sour, etc.) A total of 70 common drug/disease targets were discovered, including CEBPA (CCAAT/Enhancer-Binding Protein Alpha), CPT2 (Carnitine Palmitoyltransferase 2), CRP (C-reactive protein), ICAM1 (Intercellular Adhesion Molecule 1), IL6 (interleukin 6), and PECAM1 (Platelet And Endothelial Cell Adhesion Molecule 1), and proteins such as EGFR, TNF, ITGAL, etc. were at key nodes in the PPI network.

The above genes may be a novel target for GBS treatment. For example, the relationship between TNF and GBS has been reported in a number of studies, showing that it is highly related to the inflammatory response of GBS, and there have been case reports claiming that anti-TNF therapy may trigger the occurrence of GBS (22). All types

of GBS have been reported to have increased serum levels of TNF-α (23,24). There are some evidences that EGFR and ITGAL are directly involved in GBS; in particular, the EGFR signal plays an important role in demyelination and remyelination e, which may underpin a part of the mechanism of GBS development. Thus, anti-EGFR therapy may have a potential therapeutic value. Also, ITGAL is associated with multiple sclerosis (25). It is possible that these mechanisms (similar to demyelination-like effects, remyelination, multiple sclerosis) mediate the occurrence of GBS. The CREBBP gene is ubiquitously expressed and involved in the transcriptional co-activation of many different transcription factors. It binds to cAMP-response element binding protein (CREB), this gene is now known to play critical roles in embryonic development, growth control, and homeostasis by coupling chromatin remodeling to transcription factor recognition. The direct influence of CREBBP on GBS is still unknown. A study used quantitative global gene expression microarray and analyzed the peripheral blood leukocytes samples of 7 GBS patients with and 7 healthy controls (26). They found that CREBBP was significantly increased (fold change =2.56, P=0.034), and they also proved that CREB (the target of CREBBP) is one of the important hub genes. CPT2 is a nuclear protein which is transported to the mitochondrial inner membrane. The variation of CPT2 may cause symptoms like recurrent myoglobinuria, episodes of muscle pain, stiffness, and rhabdomyolysis (27,28), which are consistent with manifestation of GBS. Moreover, it was reported that GBS and CPT2-deficiency associated increase of rhabdomyolysis episodes are both statin-induced muscle symptoms. However, the definite causal relationship between GBS and CPT2 needs further investigation. Collectively, many well-known targets, and especially inflammatory roles, may be involved in the therapeutic mechanism of the GBS-EP and the pathogenesis of GBS, and are worthy of in-depth follow-up study.

In GO enrichment analysis, the enrichments of biological processes and cellular components were the most significant, such as GO:0006954-inflammatory response, GO:0005615 extracellular space, GO:0010628 positive regulation of gene expression, GO:0097191 extrinsic apoptotic signaling pathway, GO:0045944 positive regulation of transcription from the RNA polymerase II promoter, GO:0045121 membrane raft, GO:0009897 external side of plasma membrane, GO:0031663 lipopolysaccharide-mediated signaling pathway, GO:0051092 positive regulation of NF-kappaB transcription factor activity, GO:0043066-negative regulation of the apoptotic process, and GO:0006955-immune response, etc. This is consistent with the established etiological and pathological phenotypic characteristics of GBS (29-33).

In the signaling pathways, influenza A, hepatitis B, malaria, and hepatitis C were highly enriched. Interestingly, this result implied that the herbs in the GBS-EP, with excellent activity of "strengthening the Zheng-Qi and clearing away the Heat-Evil" in the TCM theory, can target similar roles to the pathogenesis of the hepatitis B, hepatitis C, influenza A, malaria, etc. This is reasonable as the pathogenesis of GBS can include abnormal immune response caused by infectious pathogens. On the other hand, clinical evidence also shows that GBS can be induced after injection of the influenza A (34-37) and hepatitis B (38-40) vaccines. Moreover, malaria and GBS have also been reported to be comorbidities (41-43). The enrichment results of the above signaling pathways help in understanding the pathogenesis of GBS and also in discovering potential treatments for GBS from the aforementioned comorbid phenomena. In addition, we noticed some unseen enrichment of signaling pathways for GBS, such as hsa04068: FoxO signaling pathway, hsa05321: inflammatory bowel disease (IBD), etc. These signals are highly novel and are worthy of further exploration.

Based on above analysis, the results can help guide clinical practice of GBS treatment. For example, some hub genes may play more important roles than others (e.g., EGFR, TNF, and ITGAL), and the proportion of the ingredients/herbs (such as licorice, atractylodes, and cork) associated with these genes can be increased to test a potential performance optimization our hospital's experiential prescription. Moreover, our experiential prescription may alternatively play a role when some drugs cannot be given in some scenarios. For example, IL-6 is a common target of many herbs in this prescription and a GBS relative gene. For some patients cannot receive the IL-6 antibody treatment for social or medical environmental reasons, this prescription can be used as a preferred choice. We believe that the outcomes of this work may help conduct drug innovation through the network pharmacology analysis. However, there are some difficulties at present. For example, the content of specific ingredients of each drug is not fully understood. In addition, currently, the oral bioavailability of each ingredient was obtained only through the database, and there is no actual animal or clinically validating data. In addition, the level of the specific targets of these ingredients in the key pathological

tissues and cell types of GBS still needs to be identified.

Still, this study has some limitations. The major limitation is that it does not include the laboratory verification of some important molecules, such as EGFR, TNF, and ITGAL. Moreover, some proteins within the common-target set might be detectable in the blood sample using ELSIA or Cytokine chips, such as IL6, TGFB1, TNF-α, VEGFA, MMP9, CXCL10, IL10, IL1A, CCL3, and ICAM3. Our further work will accumulate sufficient clinical samples to provide conducive evidence (by dividing individuals into the healthy control, GBS, and GBS-treated-by-TCM). Besides, we mainly focused on the level of gene expression, but the correlation between metabolome changes and the key ingredients are not clear.

In summary, this study used network pharmacology to analyze the mechanism of the hospital GBS-EP in GBS treatment. The 154 active ingredients (such as quercetin, dehydrotanshinone IIA, stigmasterol, etc.) may target key genes (such as EGFR, TNF, and ITGAL, etc.) and play a therapeutic role through crucial functions, like the inflammatory response, extracellular space, and positive regulation of gene expression, and signaling pathways, such as influenza A, hepatitis B, malaria, and hepatitis C.

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Footnote

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