

Molecular Network Analyses of Acupuncture Treating Bronchial asthma Based on Bioinformatic Approaches

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ABSTRACT

Aim: To holistically analyze the acupuncture anti-asthmatic mechanism based on bioinformatics analysis and text mining.

Methods: Literature searching across 7 databases restricted in the past decade regarding acupuncture treating asthma, text mining, the association network of meridian-acupoint-gene, hub genes and core acupoints, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analysis as well as 1 significant KEGG pathway visualization were performed by Cytoscape and R softwares.

Results: 30 studies with 71 genes were incorporated for bioinformatics analyses. The topological analysis of meridian-acupoint-gene network showed top 20 hub genes included immunoglobulin heavy constant epsilon (IGE), CD4 molecule (CD4), CD8a molecule (CD8), surfactant protein A1 (SFTPA1), interferon gamma (IFNG), interleukin 10 (IL10) and IL17A etc. The core acupoints were Feishu (BL13), Dazhui (GV14) and Fengmen (BL12). The significantly enriched GO terms were positive regulation of lymphocyte activation, regulation of immunoglobulin production etc. The enriched KEGG pathways included Asthma, T cell receptor signaling, IL-17 signaling, T Helper 1 (Th1) and Th2 cell differentiation etc. The visualized Asthma pathway contained 7 enriched genes.

Conclusion: The holistic networks of acupuncture-related anti-asthmatic meridian-acupoint-gene association and molecular mechanism were revealed by our data mining plus bioinformatics analysis, suggesting the current focus on the reversion of Th1/Th2, Th17/regulatory T (Treg) cells imbalanced ratio and anti-inflammatory action of acupuncture.

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INTRODUCTION

Bronchial asthma is a chronic respiratory disease characterized by intermittent wheeze with concomitant sputum sound, as well as dyspnea and expiratory airway obstruction.¹ The sufferers are among different ages, ethnicities and races. It causes a tremendous burden to the families, society and globe.² The mainly conventional treatments include inhaled corticosteroids, long-acting β -agonists, and asthma biologics. But the problems of adverse effects, drug dependence, and drug costs impede the adherence to western medicine.^{3,4} leading to the uncontrolled asthma associated with increased burdens of morbidities and mortalities. Finding the alternative and complementary options are of importance for those asthmatic patients.

Acupuncture is increasingly applied in clinical practice to treat various diseases like asthma, and its cost-effectiveness evaluation is acceptable and satisfactory.⁵ So far, different clinical and basic studies have been reported in terms of acupuncture treating asthma, the mechanisms involve mostly the investigation of the pure change of biomarkers, and a single pathway. During recent years, as the development of microarray, proteomics, metabolome and network biology, emerging research prove that our human body is regulated by molecular network synergistically helping the inner circumstance recover and heal during the acupuncture treatment of a disease.⁶⁻⁹ Under this context, the bioinformatics analysis based on the published data and professional databases is approved and popular to systematically analyze the underlying principle and law of target therapy^[9]. Whereas, analysis of the holistic mechanism of acupuncture treating asthma based on bioinformatics tools and text mining of the published literature remains unclear.

Hence, we planned to approach the bioinformatics analysis by 3 steps: (1) Literature review and text mining: we screened the published literature focusing on acupuncture treating asthma over the

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past decade, through text mining, we collected the related meridians, acupoints and gene biomarkers; (2) Construction of meridian-acupoint-gene interaction network using Cytoscape software and identification of the hub genes by means of CytoNCA plugin; (3) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analysis as well as 1 significant KEGG pathway visualization.

METHODS

Acquisition of Eligible Literature Data

We searched the Chinese databases including CNKI, VIP and Wanfang (from 2012 to November 15, 2021), and the English databases subsuming PubMed, Web of Science, Science Direct and Ovid (publication time filter:10 years). The searching terms were acupuncture, asthma, needling or bronchial asthma (Supplementary 1 for detailed search strategy of each database).

Inclusion Criteria

(1) Clinical and animal studies about acupuncture treating asthma with biomarker changes; (2) Acupoint with specific location; (3) Literature should be published in the Chinese core journals to improve the credibility.

Exclusion criteria

(1) No independent acupuncture therapy; (2) Meta-analysis, review and case report; (3) Main interventions are moxibustion, auricular acupuncture, dry needling, acupoint paste, acupoint massage and acupoint injection. With respect to duplicate literature, we included the first version.

After the eligible papers were obtained, we retrieved the research information like title, author, country, year, subject, group, acupoint, meridian, duration and biomarkers, into an Excel sheet to serve as the original database.

Construction of meridian-acupoint-gene interaction network

We associated the meridians with the relative acupoints, along with acupoints to the corresponding gene targets. The gene symbols used in different studies may vary, so we put them into UniProt (<https://www.uniprot.org>) and Gene databases (<https://www.ncbi.nlm.nih.gov/gene>) for standardization, followed by deletion of duplicate gene symbols to ensure the genes unique for subsequent analysis. After determination of the correlation of merian-acupoint and acupoint-gene, we imported the interaction network information into Cytoscape software (version 3.9.0). Cytoscape is an open source software for visually integrating biomolecular interaction networks with high-throughput expression profiles, functional annotations and other molecular states [10]. After setting the property of nodes, Cytoscape plugin CytoNCA was selected to analyze the hub nodes. CytoNCA is an excellent tool to detect essential nodes in biological networks, via integrating biological data with topological data [11]. The centrality related topological parameters comprise “betweenness”, “closeness”, “degree”, and “network” etc. According to one previous study [9], “degree” in topology structure can be one determinant factor of hub genes. “Degree” refers to the number of edges to a

node, the more the “degree” is, the more crucial the node is [12]. Here we chose “degree” to identify the top 20 as the hub genes for further KEGG and GO enrichment analysis.

KEGG and GO Enrichment Analysis

We used statistical packages installed in R software (version 4.1.1) for GO and KEGG pathway enrichment analysis. [13] The “org.Hs.eg.db” package was used for turning the gene symbols to entrezIDs for followed analysis. Thereafter, “clusterProfiler”, “ggplot2” and “enrichplot” packages were adopted to seek the enriched GO items and KEGG pathways, [14, 15] where p and q values were both set as $\alpha=0.05$ to indicate the significance. GO enrichment terms included biological process (BP), cellular component (CC), and molecular function (MF). Top 10 enriched GO terms and KEGG pathways were presented by bubble plots. Additionally, we employed the “pathview” package for visualizing one key pathway with enriched genes marked specifically. [16]

RESULTS

Eligible data Identification

After systematically reviewing the literature, we finally identified 30 studies [17-46] for bioinformatics analysis. The general profiles of the included research were displayed in Supplementary Table 1. The flowchart of our study is shown in Fig.1. All the eligible studies were conducted in China, among which 2 were clinical trials, along with 9 studies compared verum acupuncture vs. sham controls. After collection and matching to the UniProt and Gene databases, a total of 71 genes were actually listed as the gene symbols for further analysis (Supplementary 2).

Table 1: Degrees outline of 20 hub genes and acupoints

<i>Gene</i>	<i>Degree</i>	<i>Acupoint</i>	<i>Degree</i>
IGE	9	Feishu (BL13)	72
CD4	9	Dazhui (GV14)	66
IGG	9	Fengmen (BL12)	57
IGM	9	Zusanli (ST36)	19
CD3	9	Shenshu (BL23)	7
CD8	9	Danzhong (CV17)	7
SFTPA1	8	Dingchuan (EX-B1)	7
IL17A	5	Jianshi (PC5)	7
IL4	5	Zhigou(TE6)	7
IFNG	5	Taixi (KI3)	7
IL5	4	Tianshu (ST25)	2
TGFB1	4	Shangjuxu (ST37)	2
IL10	4	Chize (LU8)	2
CCL5	3	Kongzui(LU6)	2
CCL11	3	Lieque (LU7)	2
IL13	3	Quchi (LI11)	2
SLC3A2	3	Hegu(LI4)	2
ATP1A3	3		
IL9	3		
IL25	3		

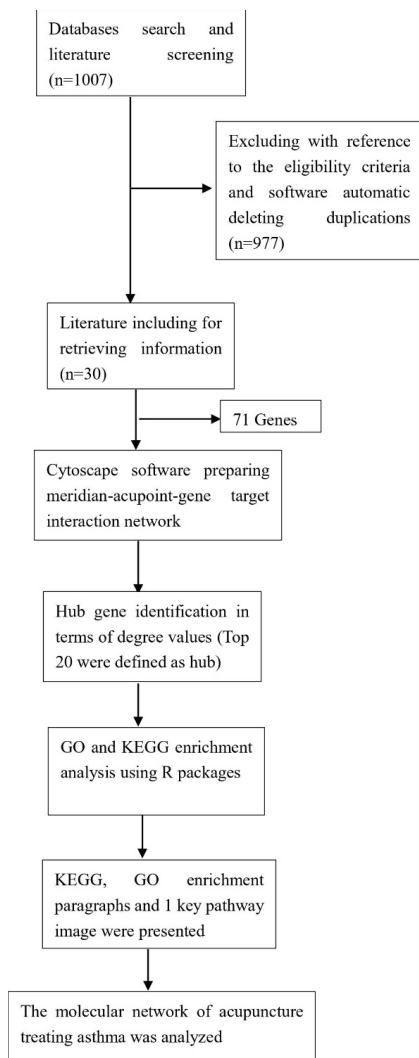


Fig.1: The research flowchart

Meridian-acupoint-gene Targets Interaction Network Preparation and Hub Gene Detection

We imported the necessary information into Cytoscape software, including the network working table and nodes type table. After adjusting the basic attributes, the meridian-acupoint-genes interaction network was acquired as Fig.2. The meridians contain Lung Meridian of Hand-Taiyin, the Stomache Meridian of Foot-Yangming, the Urinary Bladder Meridian of Foot-Taiyang, The Large Intestinal Meridian of Hand Yangming, The Kidney Meridian of Foot-Shaoyin, and Triple Energizer Meridian of Hand Shaoyang etc (Supplementary 2). The acupoints involve Feishu (BL13), Dazhui (GV14), Fengmen (BL12), Zusanli (ST36), Shenshu (BL23), and Danzhong (CV17) etc. (Supplementary 2). Then we used the CytoNCA plugin to calculate the degree value of each node, turning out to be 98 nodes, consisting of 10 meridians, 17 acupoints, 71 gene symbols, and 270 degrees in total (Supplementary 2). Top 20 genes were determined as hub genes (Table 1). The top 3 acupoints used most were Feishu (BL13), Dazhui (GV14) and Fengmen(BL12), belonging to the acupoints of Urinary Bladder Meridian of Foot-Taiyang and Governor Meridian. These 3 acupoints were frequently used in combination.

GO and KEGG Enrichment Results

Following the hub genes ensured, we utilized the R packages such as “org.Hs.eg.db”, “clusterProfiler”, “ggplot2” and “enrichplot”, to perform the GO and KEGG enrichment analysis. It revealed that top 10 enriched BP terms of GO were positive regulation of lymphocyte activation, positive regulation of leukocyte activation, positive regulation of cell activation etc. (Table 2). The top 10 enriched BP, CC and MF terms in GO were displayed in Fig.3. The top 10 enriched KEGG pathways were shown in Fig.4. The information of top 10 KEGG

Table 2: Information of top 10 enriched BP terms of GO

ONTOLOGY	ID	Description	Q value	Gene symbols	Count
BP	GO:0051251	positive regulation of lymphocyte activation	1.01E-10	IGHE/CD4/CD40LG/IL4/IFNG/IL5/TGFB1/IL10/CCL5/IL13	10
BP	GO:0002696	positive regulation of leukocyte activation	1.45E-10	IGHE/CD4/CD40LG/IL4/IFNG/IL5/TGFB1/IL10/CCL5/IL13	10
BP	GO:0050867	positive regulation of cell activation	1.45E-10	IGHE/CD4/CD40LG/IL4/IFNG/IL5/TGFB1/IL10/CCL5/IL13	10
BP	GO:0002637	regulation of immunoglobulin production	3.75E-09	CD40LG/IL4/IL5/TGFB1/IL10/IL13	6
BP	GO:0002700	regulation of production of molecular mediator of immune response	4.47E-09	CD40LG/IL17A/IL4/IL5/TGFB1/IL10/IL13	7
BP	GO:1902107	positive regulation of leukocyte differentiation	4.65E-09	CD4/IL17A/IL4/IFNG/IL5/TGFB1/IL10	7
BP	GO:1903708	positive regulation of hemopoiesis	4.65E-09	CD4/IL17A/IL4/IFNG/IL5/TGFB1/IL10	7
BP	GO:0001819	positive regulation of cytokine production	4.68E-09	CD4/CD40LG/IL17A/IL4/IFNG/TGFB1/IL10/IL13/IL9	9
BP	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	1.66E-08	CD4/IL4/IFNG/IL5/TGFB1/CCL5/IL13	7
B	GO:0002702	positive regulation of production of molecular mediator of immune response	1.66E-08	IL17A/IL4/IL5/TGFB1/IL10/IL13	6

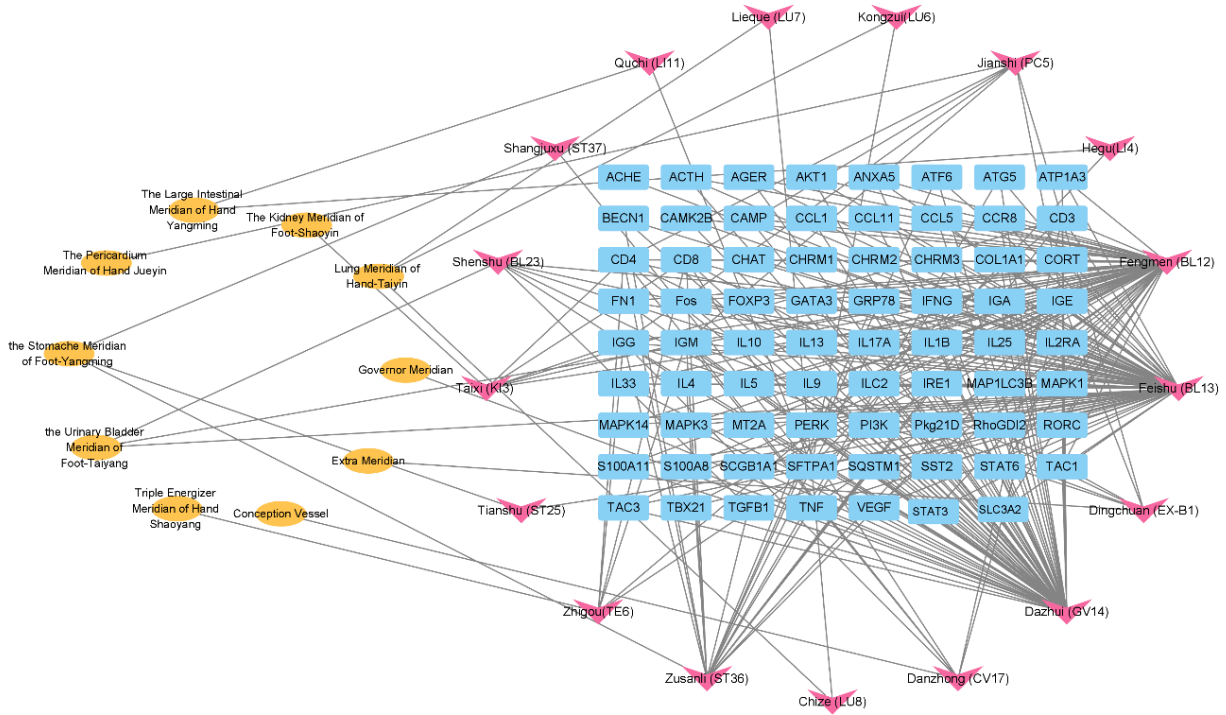


Fig. 2: The meridian-acupoint-gene targets association network. Meridians were displayed in orange, acupoints in red, and genes in blue.

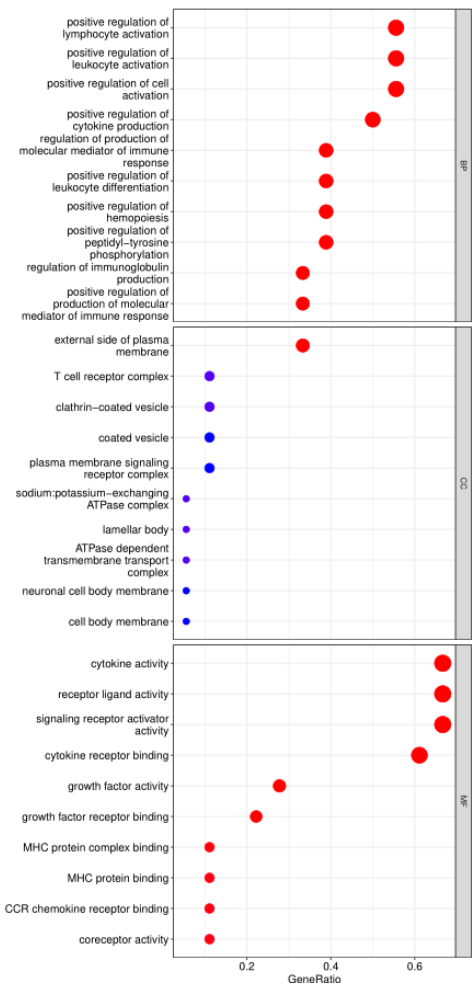


Fig. 3: The top 10 significantly Gene Ontology (GO) enrichment analysis presentation by bubble plots in 3 categories: biological process (BP), cellular component (CC), and molecular function (MF).

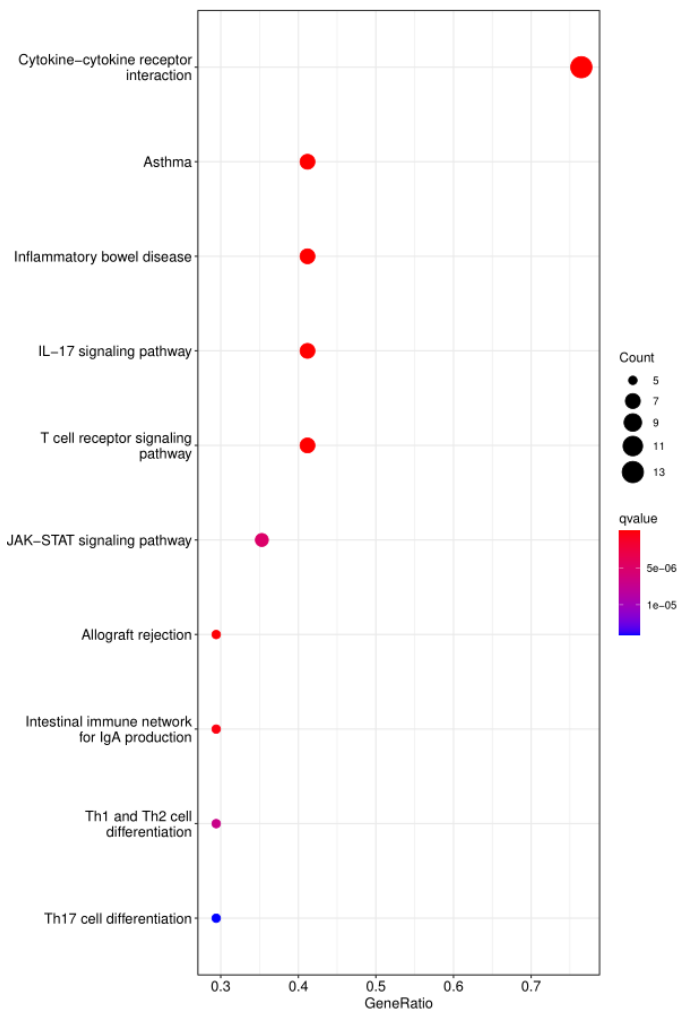


Fig. 4: The top 10 significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway presented by bubble plots.

Table 3: Information of top 10 enriched KEGG pathways

ID	Description	qvalue	geneID	Count
hsa04060	Cytokine-cytokine receptor interaction	2.11E-14	CD4/IGM/IL17A/IL4/IFNG/IL5/TGFB1/IL10/CCL5/CCL11/IL13/IL9/IL25	13
hsa05310	Asthma	3.67E-12	IGM/IL4/IL5/IL10/CCL11/IL13/IL9	7
hsa05321	Inflammatory bowel disease	6.24E-10	IL17A/IL4/IFNG/IL5/TGFB1/IL10/IL13	7
hsa04657	IL-17 signaling pathway	6.66E-09	IL17A/IL4/IFNG/IL5/CCL11/IL13/IL25	7
hsa04660	T cell receptor signaling pathway	1.09E-08	CD4/IGM/CD8/IL4/IFNG/IL5/IL10	7
hsa05330	Allograft rejection	1.14E-07	IGM/IL4/IFNG/IL5/IL10	5
hsa04672	Intestinal immune network for IgA production	3.68E-07	IGM/IL4/IL5/TGFB1/IL10	5
hsa04630	JAK-STAT signaling pathway	5.02E-06	IL4/IFNG/IL5/IL10/IL13/IL9	6
hsa04658	Th1 and Th2 cell differentiation	6.99E-06	CD4/IL4/IFNG/IL5/IL13	5
hsa04659	Th17 cell differentiation	1.40E-05	CD4/IL17A/IL4/IFNG/TGFB1	5

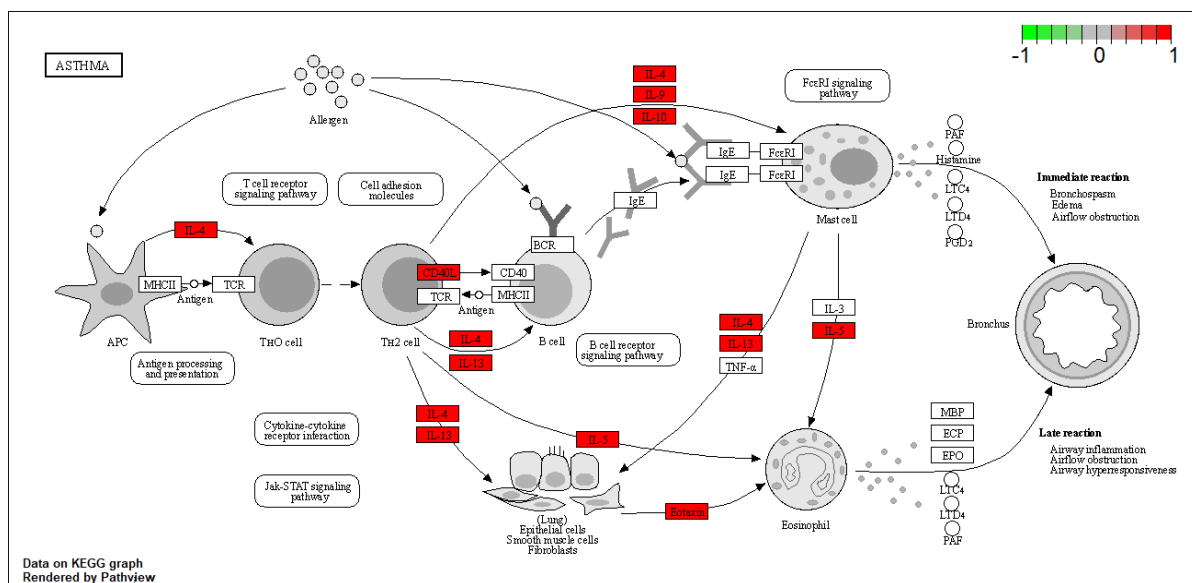


Fig. 5: The significantly enriched Asthma pathway of interest was visualized along with screened genes marked in red.

pathways were listed in Table 3, including Cytokine-cytokine receptor interaction and Asthma pathways. The “pathview” R package was adopted to visualize the Asthma pathway (KEGG ID: hsa05310) with enriched genes labelled in red (Fig.5).

DISCUSSION

Bronchial asthma is a commonly chronic airway inflammatory disease with acute episodes induced by various risk factors like allergen, afflicting around 1% to 18% of the population among different countries [1, 47]. Due to the potential advantages of acupuncture managing asthma, the world health organization recommends acupuncture treatment as an adjunctive for asthma control [17]. Here in our bioinformatic network analysis, we find that in the current research, the focus with regard to acupuncture against asthma is on the immune system regulation and the anti-inflammatory action of acupuncture. The enriched GO terms were like positive regulation of lymphocyte activation, regulation of immunoglobulin production etc. The enriched KEGG pathways included Cytokine-cytokine receptor interaction, Asthma, IL-17 signaling pathway and T cell receptor signaling pathway etc.

In terms of cellular and molecular mechanism exploration, traditional approaches generally focus on a single pathway or some genes, proteins expression changes. Whereas,

nowadays is a mass data era, as the rapid advancements of mass spectrometry, microarray, proteomics technologies etc., combined with the developments of computational and bioinformatics platforms, biomedical data analysis based on bioinformatics methods are quite popular over the past years. Bioinformatics analysis is an efficient technique to integrate a complex biological system and resource as a holistic unit, thus making it possible to probe the comprehensive mechanisms behind diseases and treatments.^{48, 49}

As for Chinese medicine, the combination of network pharmacology and network biology has developed as an increasingly valuable approach to transfer the “one target, one drug” research paradigm into “network target, multi-components” mode, contributing to uncover the holistic mechanism of Chinese medicine.⁵⁰ Network pharmacology-based mechanism studies have been reported extensively regarding Chinese medicine treatment of asthma, and the key putative targets and active compounds were obtained effectively.⁵¹⁻⁵³ Coming to acupuncture research, the systems biology, Omics research and bioinformatics platforms also help acupuncture translate into good practice.^{54, 55} Researchers have conducted quantitative proteomics, putative gene prediction and protein interaction construction, and serial analysis of gene expression in acupuncture treating asthma studies.^{17, 23, 46}

But the bioinformatics analysis of the current mechanism trend based on the published literature remains to be reported on acupuncture treating asthma.

The respiratory system acts as the initial contact site with various pathogens. In this position, the system serves as a well-orchestrated bridge between innate and adaptive immunity^[56], among which the airways and alveolar epithelial cells are the first line of the lung immune defense. The airways of asthmatic patients manifested as infiltration of eosinophils, degranulated mast cells and lymphocytes, as well as altered epithelial cell tight junctions^[57]. Here in our work, positive regulation of lymphocyte activation, positive regulation of leukocyte activation, regulation of production of molecular mediator of immune response, were the mainly enriched topics of acupuncture treatment of asthma, which is consistent with the aforementioned primary pathology of asthma. The significantly enriched KEGG pathways involved Cytokine-cytokine receptor interaction, Asthma, IL-17 signaling pathway, T cell receptor signaling pathway, Intestinal immune network for IgA production, JAK-STAT signaling pathway, Th1 and Th2 cell differentiation, and Th17 cell differentiation etc. Asthma is driven by cytokine network, such as interleukin-4 (IL-4), IL-5, and IL-13, which trigger airway eosinophilia, mucus excessive secretion, bronchial hyper-sensitivity, and immunoglobulin E (IgE) synthesis^[58]. So we can see that acupuncture targeted cytokine network to inhibit the inflammatory and hyper-reactive progression. Among the cytokines, IL-17 signaling has been observed in different sides of asthma pathogenesis, incorporating structural changes of epithelial cells and smooth muscle contraction^[59]. Th1, Th2 and Th17 cell differentiations all were paid great attention in the asthma pathophysiology and target-therapy research. Some theories proposed that asthma induced Th2-oriented differentiation of T lymphocytes more than Th1-oriented differentiation, which resulted in the imbalance of Th1/Th2 ratio, latter aggravating asthma condition^[60, 61]. Likewise, Th17 cells/regulatory T cells (Tregs) ratio is also the key role in the evolution of asthma. Tregs were reported to inhibit the inflammatory reaction of Th2 cells in asthma attenuation. The main effector component of Th17 cells embodies as the secretion of IL-17, which is the crucial pathological driver of asthma onset^[59, 62]. Altogether, reducing T-cell activation or increasing the Th1/Th2 or Treg/Th17 ratio might be beneficial for the asthma control from the stump^[61]. Additionally, JAK/STAT signaling pathway participates in mediating Th17 cell differentiation, survival and proliferation^[63]. The current literature reflect that acupuncture could positively regulate the Th1/Th2, Treg/Th17 ratio, along with T cell receptor signaling pathway possibly via JAK/STAT signaling, so as to treat asthma.

From our literature mining, we find that current acupuncture studies on asthma mostly focus on a single pathway and a few genes alterations, comparatively, the omics research and study integrated with bioinformatics is sparse, which awaits more exploration. The limitation of our study lies mainly in the pure acupuncture intervention we choose, which is partly against the true clinical acupuncture practice. The abundant treatment prescriptions of Chinese medicine deserve extensive research from different aspects.

CONCLUSION

In summary, our study systematically analyzed the molecular network of acupuncture treating asthma based on text mining and bioinformatic resources, suggesting the frequently used acupoint combination is BL13, GV14 and BL12, and acupuncture-relevant anti-asthmatic mechanisms focus mainly on regulation of genes like CD4, IGM, IL17A, IL4, IFNG and IL10 in pathways Asthma, T cell receptor signaling, IL-17 signaling, Th1 and Th2 cell differentiation etc. Our data mining plus bioinformatics analysis appeared to be a useful method to construct the association network of meridian-acupoint-gene, and uncover the underlying mechanism.

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Authorship Contributions

Haizhen Zheng: Methodology, Data cleaning, Original draft preparation; Ruihua Nie: Conception, Bioinformatics analysis and paper revision, correspondence.

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