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Xiaoqinglong decoction suppresses childhood cough variant asthma and inhibited the body inflammatory response by regulating IL-6/STAT3 signalling pathway

Yuzhe Ren, PhD[a,c], Xing Li, PhD[b], Yuanjie Zhang[d], Zilong Yan, PhD[e,*]

Abstract

Xiaoqinglong decoction (XQLD) is widely used clinically in the treatment of childhood cough variant asthma (CVA). However, its potential mechanism is still unknown. In the present study, the authors investigate the biological network and signalling pathway of XQLD in treatment of childhood CVA using network pharmacology-based analysis and experimental validation. By using the Bioinformatics Analysis Tool Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) database, the authors confirmed the correlation between XQLD and asthma, and the authors screened 1338 potential target genes of Mahuang and Guizhi, the most active herbs in XQLD. By overlapping "Childhood asthma-related genes" of DisGeNET database, the authors identified 58 intersecting genes of Childhood asthma and 1338 target genes of Mahuang and Guizhi. The intersecting genes were used to construct the protein-to-protein interaction and performed Gene Ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses. Gene Ontology enrichment analysis demonstrated 359 Biological Process terms, 16 Cellular Component terms, and 26 Molecular Function terms. Meantime, 75 terms of Kyoto Encyclopedia of Genes and Genomes signalling pathway were involved in enrichment analysis. These candidates showed a significant correlation with inflammatory response and positive regulation of tyrosine phosphorylation of STAT protein. In addition, XQLD treatment significantly upregulated serum interferon-γ expression, and downregulated serum interlukin-6 expression of CVA mice. XQLD treatment significantly inhibited phosphorylation of STAT3 in bronchial-lung tissues. Our data suggest that XQLD effectively alleviated bronchial-lung tissue damage in CVA mice and inhibited the body inflammatory response by regulating interlukin-6/STAT3 signalling pathway.

Keywords: Child cough variant asthma, IL-6, pharmacology network, STAT3, Xiaoqinglong decoction

Introduction

Cough variant asthma (CVA) is a type of asthma in which the main symptom is a dry, non-productive cough. People with CVA often have no other "classic" asthma symptoms, such as wheezing or shortness of breath[1,2]. The original definition of CVA was described by Glauser in 1972, and subsequently renamed by McFadden in 1975, and Corrao and colleagues in 1979[1,3,4], as 'cough variant asthma' cough can be the sole presenting symptom. In China, CVA is the most common cause of clinical chronic cough (32.6%), and it is also the leading cause of chronic cough in children, which seriously affects people's daily life[5–7]. The current combined treatment of western medicine can alleviate the seasonal onset of CVA patients to a certain extent, relieve airway spasm and control airway inflammatory reaction[8]. In adults with CVA, traditional Chinese medicine (TCM) herbal medicine results in improved cough frequency and severity scores compared with western medicine Montelukast[9]. However, for children therapy, the efficacy of drug was limited by the contraindications

HIGHLIGHTS

- Network Pharmacology identified active ingredients and potential targets of Xiaoqinglong decoction (XQLD).
- XQLD alleviated inflammation of lung tissues in cough variant asthma mice model.
- XQLD targeted Childhood asthma by interlukin-6/STAT3 signalling pathway.

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and side effects. Therefore, development of new therapeutic strategy with fewer side effects is necessary.

Xiaoqinglong decoction (XQLD) is a TCM including Ganjiang (Zingiberis Rhizoma), Mahuang (Ephedrae Herba), Guizhi (Ramulus Cinnamomi), Baishao (Radix Paeoniae Alba), Gancao (Glycyrrhizae Radix et Rhizoma), Xixin (Asari Radix Et Rhizoma), Banxia (Pinelliae Rhizoma) and Wuweizi (Schisandra chinensis Fructus)[10]. XQLD has been used widely in treatment of respiratory diseases in China, due to its effect of anti-oxidation, anti-inflammation, and anti-apoptosis[11,12]. In addition, XQLD modified and Subtracted Recipe is widely used clinically in the treatment of children's CVA[13]. Several studies have proved therapy efficiency of XQLD in chronic obstructive pulmonary disease (COPD) and asthma[9,11]. However, pharmacodynamic effect and molecular mechanism of XQLD is still unclear. Clarifying the molecular mechanism of XQLD in treating childhood CVA, to provide scientific basis for the combined use of XQLD and Western medicine.

In the present study, we explored the potential of XQLD in asthma treatment. We investigated the functional effect of XQLD by using network pharmacology. We analyzed the most active ingredients Mahuang and Guizhi in XQLD. Herb-ingredient analysis identified 1338 union target genes. By overlapping "Childhood asthma-related genes" of DisGeNET, we screened 58 intersection targets as potential targets of XQLD for treatment of childhood asthma. The 58 candidates were thus used in PPI network analysis and the protein interaction relationship was obtains accordingly. Gene Ontology enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed these targets were significantly correlated to the pathways of Cytokine activity, Tyrosine phosphorylation of STAT protein and Inflammatory response. Finally, drug treatment of established CVA mice model demonstrated XQLD effectively suppressed the symptoms of CVA by regulating interlukin-6 (IL-6) and STAT3 phosphorylation compared to the dexamethasone treatment (Fig. 1).

Materials and methods

CVA lung tissues and ethical approval

The mice were euthanized and the bronchial-lung tissues were collected. Tissues were embedded, sliced and stained, and sections were observed using an optical microscope (Dmi 1, Leica, Germany). All animal experiments were carried out in accordance with the Experimental Animal Welfare Act and approved by Animal Experimentation Ethics Committee of our institution (Longgang District, Shenzhen, China) registered number: SZLDH2019LSYA-074, registered date: 2019.8.1). The work has been reported in line with the ARRIVE criteria[14].

Bioinformatics Analysis Tool Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM) bioinformatics

The human genes associated with XQL decoction were acquired from the BATMAN-TCM (http://bionet.ncpsb.org.cn/batman-tcm/) database. The online bioinformatics analysis tool specially designed for studying the molecular mechanisms of TCM, and is based on TCM ingredients' target prediction[15].

XQLD preparation

XQLD was prepared according to the method reported before[12,16]. Sixteen grams of Guizhi, 14 g of Mahuang, 18 g of Wuweizi, 18 g of Ganjiang, 14 g of Baishao, 18 g of Xixin, 54 g of Banxia and 36 g of Gancao were obtained at the pharmacy of our insititution. These herbs were macerated in 1600 ml of water for 3 h at room temperature. Then the mixture was boiled until the total volume reduced to 500 ml. All extracts then were stored at 4°C.

Animal experiments

CVA mice model of asthma was established as described before[17]. All male BALB/C mice aged 6–8 weeks old were raised in specific pathogen-free level animal laboratories of the animal centre in our institution, with a room temperature of 22–25°C and a relative humidity of 50–60%. They ate and drank freely, alternating between light and darkness for 12 h. Then mice were randomly divided into four groups ($n = 5$ per group): Control group, CVA group, CVA dexamethasone group, and CVA XQLD group. After 1 week of accustomization, except control group, the rest of mice were intraperitoneally injected with 20 μg ovalbumin (OVA; Aladdin Regents, Shanghai, China) to induce allergic lung inflammation. The mice were administrated on dexamethasone (gavage, 1 mg/kg; HY-14648, MCE, China), XQLD (gavage, 7.5 g/kg) for 21 days. Following all animal treatments, peripheral blood and lung tissues were collected. All mice were anaesthetised and decapitated to obtain lung tissues. Part of tissue was fixed in 4% paraformaldehyde and the others were snap-frozen with liquid nitrogen and stored at – 80°C for total protein extraction.

Enzyme-linked immunosorbent assay (ELISA)

Peripheral blood samples were taken from the mice. Serum samples were stored at -80 degrees until analyzed. Serum interferon-γ (IFN-γ) and IL-6 levels were measured by ELISA using commercial kits (RK00019 and RK00008, Abclonal, China) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (Multiskan go, Thermo). The concentration of each cytokine was calculated from the standard curve of each cytokine standard.

Western blot analysis

Total protein extracts were prepared from frozen mouse lung tissues using RIPA buffer (P0013J, Beyotime), containing proteinase inhibitor cocktail (B14001, Bimake) and phosphatase inhibitor cocktail (B15001, Bimake). Proteins were separated on 4–20% precast mini polyacrylamide gels (SurePAGE, GenScript) and transferred to polyvinylidene fluoride membranes. The membranes were incubated overnight at 4°C with anti-IL-6 (A0286, Abclonal), anti-STAT3 (A1192, Abclonal), and anti-pSTAT3 (AP0715, Abclonal).

Statistical analysis

Comparisons of qRT-PCR were carried out using the Student's *t*-test. Comparisons of RNA expression in bioinformatic analysis were performed using the Wilcox test and Kruskal–Wallis test. Kaplan–Meier analyses were compared using the log-rank test. Statistical analysis was conducted using R language and GraphPad prism. *P* less than 0.05 was used to define statistical significance.

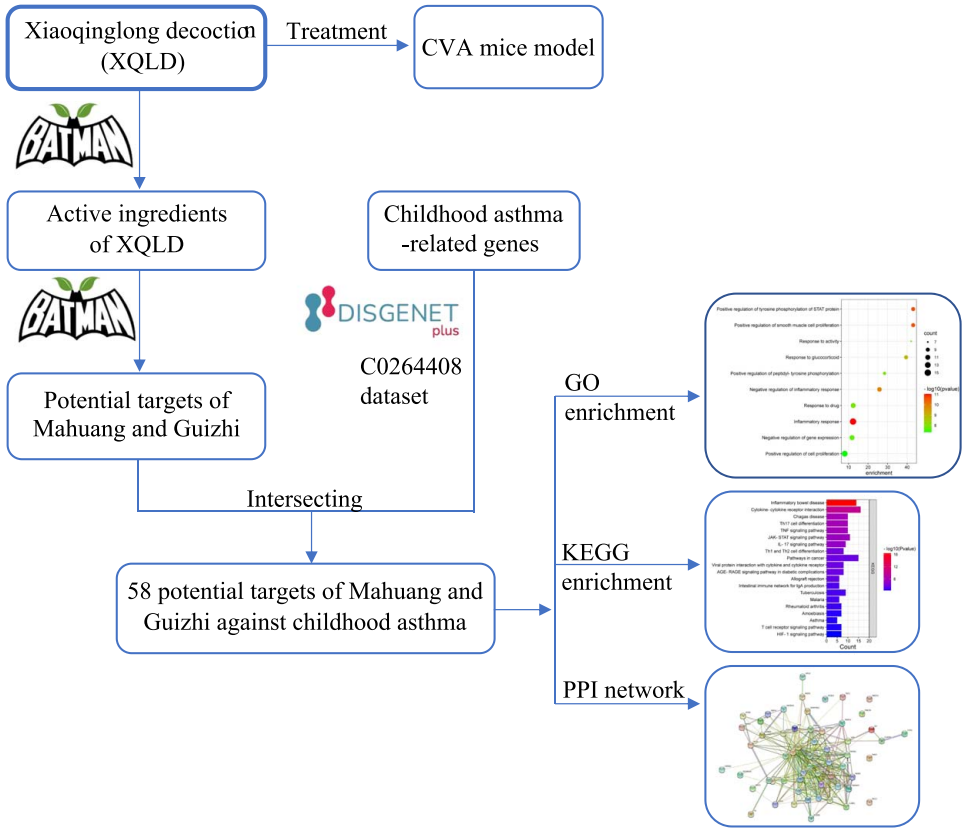


Figure 1. Research process. We adopted BATMAN-TCM and DisGeNET databases to screen the active ingredients and potential targets of XQLD. The intersecting of XQLD-targeted genes and Childhood asthma-related genes were used to construct the protein-to-protein interaction (PPI), Gene Ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of target genes. In addition, CVA mice model was established for performing drug treatment using Dexamethasone or XQLD. CVA, cough variant asthma; XQLD, Xiaoqinglong decoction.

Results

Target prediction of XQLD by bioinformatics

XQLD is a TCM composed of eight herbs, including Guizhi (Ramulus Cinnamomi), Mahuang (Ephedrae Herba), Wuweizi (Schisandra chinensis Fructus), Ganjiang (Zingiberis Rhizoma), Baishao (Radix Paeoniae Alba), Xixin (Asari Radix et Rhizoma), Banxia (Pinelliae Rhizoma), and Gancao (Glycyrrhizae Radix et Rhizoma). By using BATMAN-TCM (http://bionet.ncpsb.org.cn/batman-tcm), 741 compounds and their targets were predicted (Supplement data 1, Supplemental Digital Content 1, http://links.lww.com/MS9/A276). By "Enriched Therapeutic Target Database diseases" analysis, the target genes of XQLD were significantly correlated to Asthma. The potential capability of XQLD on treatment of Asthma and Asthma-related disease were confirmed (Table 1). Moreover, enrichment analysis results identified XQLD-targeted KEGG signalling pathways, including glycolysis/gluconeogenesis, citrate cycle (TCA Cycle), pentose phosphate, etc (Table 2).

Table 1

Enriched TTD diseases of XQL decoction targeted genes are related to Asthma.

TTD ID	Adjusted *P* value	Targets*
Analgesics	5.87e-012	55
Breast cancer	3.02e-002	30
Cardiovascular disease, unspecific	1.38e-004	30
Asthma	1.17e-003	29
Prostate	2.4e-002	27
Hypertension	4.70e-006	25
Atherosclerosis	2.20e-002	19
Pain, unspecific	5.16e-004	17
Depression	8.10e-005	17
Parkinson's disease	2.17e-002	17

TTD, Therapeutic Target Database; XQL, Xiaoqinglong.
*Adjusted *P* value smaller than 0.05.

Active ingredients and potential targets of XQLD

In the basic theory of TCM, Mahuang and Guizhi were known as the sovereign drugs of XQLD, which means the most active ingredients in the decoction. By herb-ingredient analysis, we identified the target genes of Mahuang and Guizhi respectively. Mahuang contains 157 compounds with 1274 potential target genes, whereas Guizhi contains 34 compounds with 492 potential target genes (Supplement data 2, Supplemental Digital Content 2, http://links.lww.com/MS9/A277 and 3, Supplemental Digital Content 3, http://links.lww.com/MS9/A278). And 1338 union genes of 2 herbs were identified (Fig. 2). Then, the enrich TTD diseases analysis indicated the union target genes of Mahuang and Guizhi were correlated to Asthma (Table 3) and the enriched KEGG pathways were provided (Table 4).

Table 2

Enriched KEGG pathways of XQL decoction targeted genes are related to Asthma

KEGG pathway ID	KEGG pathway name	Adjust *P* value	Targets*
has00010	Glycolysis / gluconeogenesis	1.29e-002	25
hsa00020	Citrate cycle (TCA Cycle)	1.12e-003	16
hsa00030	Pentose phosphate pathway	4.14e-002	12
hsa00071	Fatty acid degradation	9.47e-005	23
hsa00072	Synthesis and degradation of ketone bodies	2.19e-002	6
hsa00100	Steroid biosynthesis	4.49e-002	9
hsa00140	Steroid hormone biosynthesis	4.79e-004	26
has00230	Purine metabolism	1.40e-003	58
has00250	Alanine, aspartate and glutamate metabolism	4.14e-010	27
has00260	Glycine, serine and threonine metabolism	9.84e-014	32

KEGG, Kyoto Encyclopedia of Genes and Genomes; XQL, Xiaoqinglong.
*Adjusted *P* value smaller than 0.05.

Acquisition of active targets for treatment of childhood asthma from Mahuang and Guizhi

To screen the active targets of Mahuang and Guizhi against childhood asthma, we overlapped "Childhood asthma- related genes" (Supplement data 4, Supplemental Digital Content 4, http://links.lww.com/MS9/A279) from database of DisGeNET[18] (https://www.disgenet.org) with the 1338 target genes. As a result, 58 intersection targets were identified (Fig. 3).

Protein-protein interaction network analysis

The 58 intersection targets of the predicted Mahaung-Gguizhi and Childhood Asthma were imported into the STRING[19] database to select a Homo sapiens-generated PPI network map and obtain protein interaction relationships (Fig. 4).

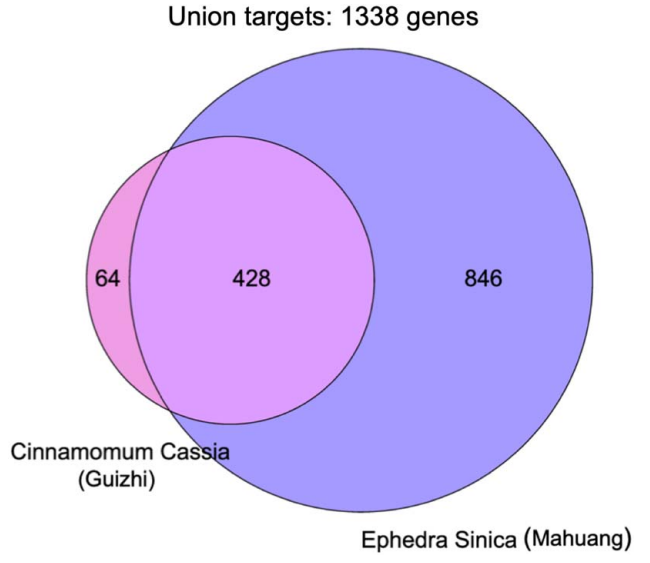


Figure 2. Union genes of Mahuang and Guizhi. Mahuang contains 157 compounds with 1274 potential target genes, Guizhi contains 34 compounds with 492 potential target genes.

Table 3

Enriched TTD diseases of Mahuang and Guizhi correlated genes are related to Asthma

TTD ID	Adjusted *P* value	Targets*
Analgesics	1.20e-012	48
Asthma	1.06e-004	26
Cardiovascular disease, unspecific	3.27e-002	20
Hypertension	3.07e-004	19
Pain, unspecific	2.68e-004	15
Pain	5.03e-004	15
Parkinson's disease	7.25e-003	15
Schizophrenia	2.88e-006	14
Heart failure	1.85e-004	14
Depression	2.12e-003	13

TTD, Therapeutic Target Database.
*Adjusted *P* value smaller than 0.05.

Gene ontology analysis and kyoto encyclopedia of genes and genomes pathway enrichment analysis

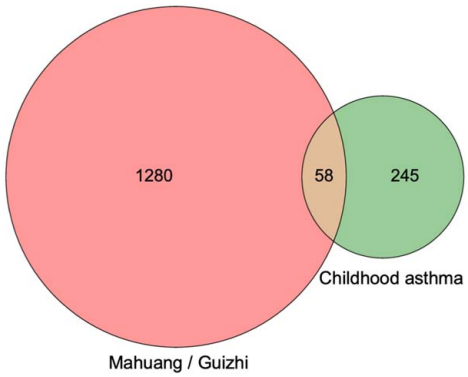
To further explore possible mechanisms of the 58 candidate targets against Childhood Asthma, we conducted Gene Ontology (GO) enrichment analysis and KEGG pathway analysis with the candidate targets by using DAVID (https://david.ncifcrf.gov/)[20,21]. The results showed that the number of biological process terms was 359, cell component was 16, and molecular function was 26. The typical biological processes. cell components and molecular functions are shown in bubble charts (Fig. 5A, B and C). Meantime, 75 terms of KEGG pathway were involved in enrichment analysis. The bar chart reflects the top 20 KEGG pathways (Fig. 5D). Notably, Cytokine activity, Tyrosine phosphorylation of STAT protein and Inflammatory response of GO pathway and Asthma, JAK-STAT, Th1, Th2, Th17 cell of KEGG signalling pathway were significantly correlated to the 58 candidate genes. Given the importance of inflammatory response and STAT protein phosphorylation on asthma progression, we speculated Mahuang-Guizhi plays a crucial inhibiting role in biological process of asthma development.

Table 4

Enriched KEGG pathways of Mahuang and Guizhi correlated genes

KEGG pathway ID	KEGG pathway name	Adjust *P* value	Targets*
has00010	Glycolysis / gluconeogenesis	1.86e-004	23
hsa00020	Citrate cycle (TCA Cycle)	1.79e-004	14
hsa00030	Pentose phosphate pathway	1.32e-003	12
hsa00071	Fatty acid degradation	4.18e-003	15
hsa00100	Steroid biosynthesis	1.25e-002	8
hsa00140	Steroid hormone biosynthesis	3.91e-006	24
has00230	Purine metabolism	3.32e-004	44
has00260	Glycine, serine and threonine metabolism	2.53e-002	12
has00270	Cysteine and methionine metabolism	6.34e-003	13
has00280	Valine, leucine and isoleucine degradation	7.76e-006	20

KEGG, Kyoto Encyclopedia of Genes and Genomes.
*Adjusted *P* value smaller than 0.05.



Overlapped 58 genes

IL17A	IL2	ACSL3	LEP
IL18R1	IL4	ARG2	LTA
SERPINE1	IL6	BCL2	MIF
SERPINE2	NAV2	CCL5	MTOR
ACE	NFKB1	CFTR	NOD2
ADIPOQ	NOS1	CRHR2	NQO1
ADORA1	PC	CRYZ	NRG1
ADRB2	PPARG	CYP11A1	PDE7B
ALOX5	TBX21	EDN1	RYR2
AREG	TGFB1	HDAC1	SCGB1A1
CYP2R1	TNF	ICAM1	SERPINB7
FCER2	TRPA1	IFNG	TET1
GC	TRPV1	IL1RN	TNFAIP3
IL10	VDR	IL2RA	
IL13	ABCC4	IL5	

Figure 3. Acquisition of active targets for treatment of childhood asthma from Mahuang and Guizhi. To screen the active targets of Mahuang and Guizhi against childhood asthma, we overlapped "Childhood asthma-related genes" from database of DisGeNET with the 1338 union target genes of Mahuang and Guizhi.

Mice model revealed functional effect of XQLD on treatment of CVA

To further validate the therapeutic effect of XQLD on CVA, we established a CVA mice model via ovalbumin stimulation as previously described[17]. The established CVA mice were divided into non-treat group, dexamethasone-treated group, XQLD-treated group. After 2 weeks of drug administration, mice were sacrificed and lung tissues, peripheral blood of mice were obtained.

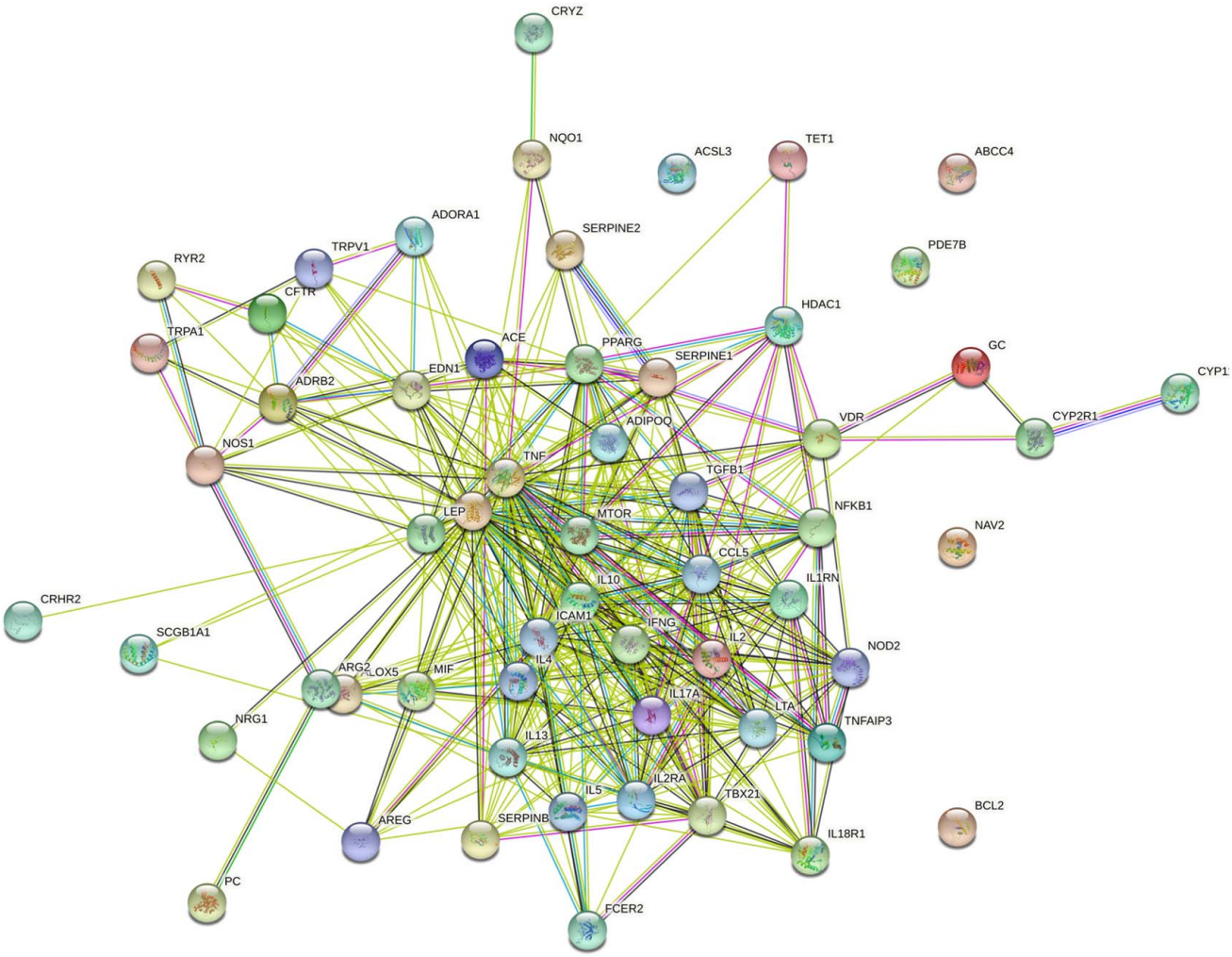


Figure 4. Protein-protein interaction (PPI) network analysis of 58 intersection targets. The 58 intersection targets were imported into the STRING database to select a Homo sapiens-generated PPI network map and obtain protein interaction relationships.

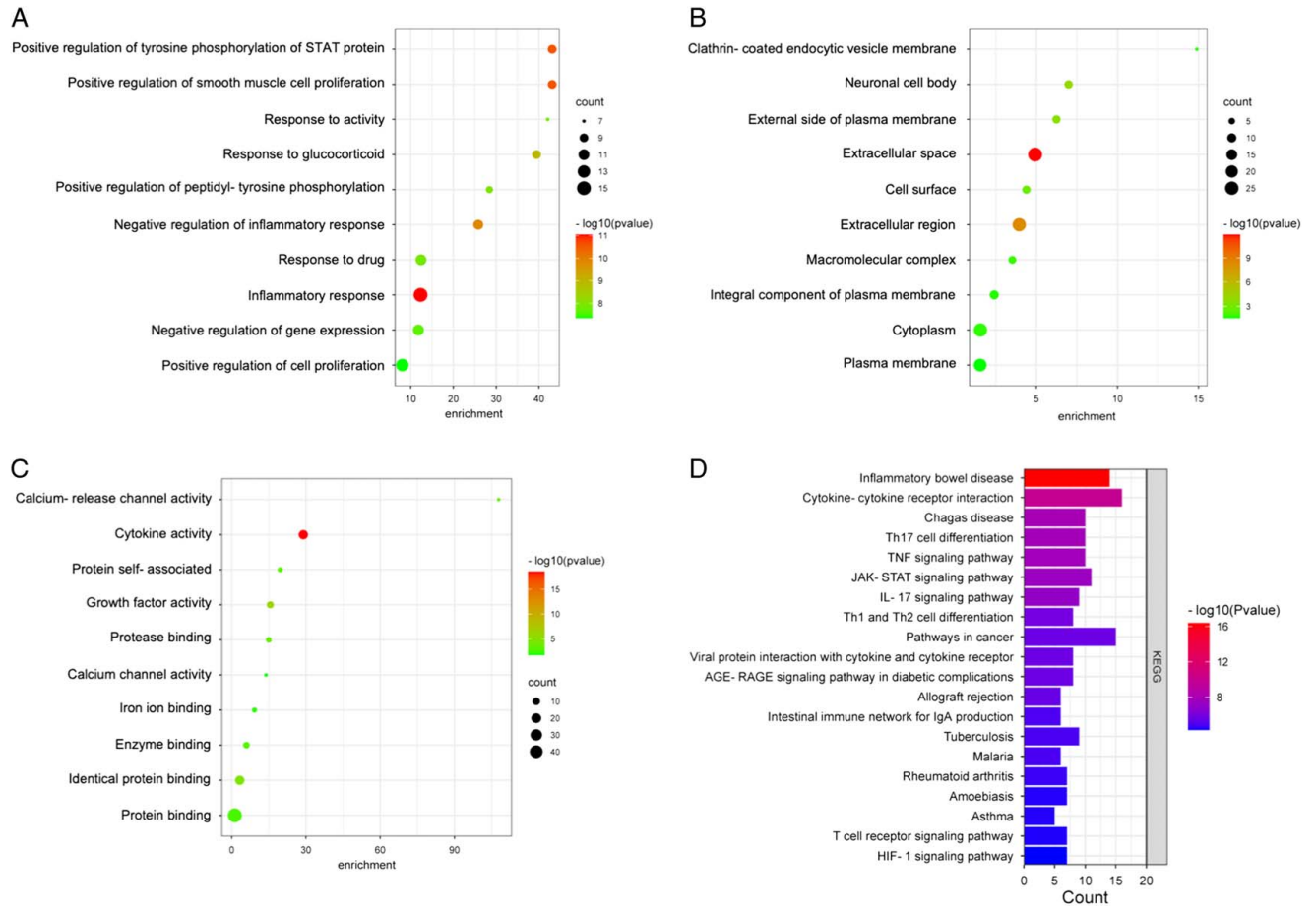


Figure 5. Mechanisms of the 58 candidate targets against Childhood Asthma. (A–C) GO analysis showed significant "Biological Process", "Cellular Component", and "Molecular Function" terms. (D) "KEGG Pathway Enrichment Analysis" showed top 20 significant KEGG pathways correlated to the 58 candidate genes. KEGG, Kyoto Encyclopedia of Genes and Genomes.

As a result, CVA mice exhibited upregulated serum IL-6 and downregulated IFN-γ expression compared to the normal control mice. Whereas the expression of serum IL-6 was significantly suppressed, IFN-γ expression levels were elevated in the dexamethasone and XQLD treatment groups (Fig. 6A). Hematoxylin and eosin staining revealed that, in comparison with the controls, there were significant inflammatory cell infiltrations in peribronchial, perivascular and alveolar and marked thickening in bronchial wall in CVA group. Administration of dexamethasone or XQLD reduced infiltration of inflammatory cells and decreased bronchial wall thickness in asthmatic mice (Fig. 6B). In addition, the levels of IL-6 and STAT3 phosphorylation in lung tissue were found significantly upregulated in CVA mice, while treatment of dexamethasone or XQLD downregulated IL-6 expression. And downregulation of STAT3 phosphorylation was only observed in XQLD-treated mice (Fig. 6C). Consistent with the bioinformatics results, the data suggested XQLD suppressed CVA by repression of STAT3 phosphorylation.

Discussion

In the present study, we investigated the relationship between XQLD and asthma treatment. We investigated the functional effect of XQLD by using network pharmacology and analyzed the most active ingredients Mahuang and Guizhi of the XQLD. By overlapping Childhood asthma-related genes, we identified 58 intersection targets as potential targets for treatment of childhood asthma. GO enrichment analysis and KEGG pathway analysis revealed these targets were significantly correlated to pathways such as cytokine activity, tyrosine phosphorylation of STAT protein and Inflammatory response. Moreover, drug treatment of established CVA mice model demonstrated XQLD effectively suppressed the symptoms of CVA by regulating IL-6 and STAT3 phosphorylation compared to the dexamethasone treatment. Our data indicated XQLD effectively alleviated bronchial-lung tissue damage in CVA mice and inhibited the body inflammatory response by regulating IL-6/STAT3 signalling pathway.

CVA is a very common subtype of bronchial asthma among children population[22–24]. The conventional western medications included glucocorticoids, antihistamine drugs, β2-agonists, and leukotriene receptor antagonists. Of those medications, leukotriene receptor antagonists have been used as the first-line treatment for such condition, and montelukast comprises the most commonly used type 1 cysteinyl leukotriene antagonist[25]. Mechanically, montelukast alleviated symptoms of CVA by regulating inflammatory factors[26]. Inhibition of inflammation and airway remodelling-related targets may become an effective

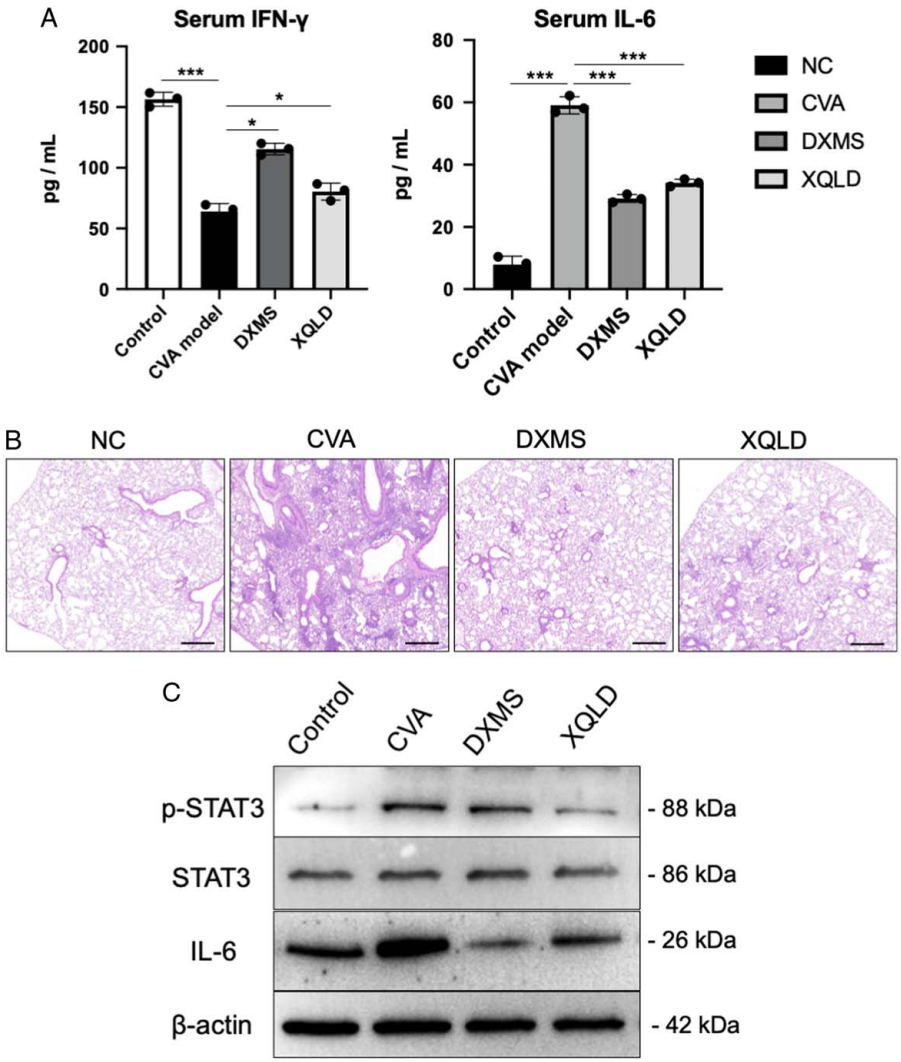


Figure 6. Mice model revealed functional effect of XQLD on treatment of CVA via IL-6/STAT3. (A) CVA mice exhibited downregulated IFN-γ and upregulated serum IL-6 expression compared to the control mice. IFN-γ expression levels were elevated, serum IL-6 was significantly suppressed in the XQLD or dexamethasone treatment groups. $^{*}P < 0.05$, $^{***}P < 0.001$. (B) Bronchial-lung tissues hematoxylin and eosin staining indicated significant inflammatory cell infiltrations in peribronchial, perivascular and alveolar and marked thickening in bronchial wall in CVA compared to the control mice. Administration of XQLD or dexamethasone reduced infiltration of inflammatory cells and decreased bronchial wall thickness in mice. (C) Expression of IL-6 and STAT3 phosphorylation in Bronchial-lung tissues were significantly upregulated in CVA mice, while treatment of XQLD or dexamethasone downregulated IL-6 expression. CVA, cough variant asthma; IFN-γ, interferon-γ; IL-6, interlukin-6; XQLD, Xiaoqinglong decoction; NC, negative control; DXMS, dexamethasone.

strategy for the treatment of CVA[27]. Moreover, regulation of immune response provides a new direction for the treatment of children CVA[28]. These studies highlighted the significance of anti-inflammatory and immune response in CVA treatment. And the alternated genes should be further investigated.

Recent years, TCM has gradually served a very important role in CVA treatment[29]. Network pharmacology analysis of TCM provided an important approach for understanding herbal formulas and predicting potential new drugs or targets[30]. Network pharmacology is a promising approach frequently applied to reveal related mechanisms and discover new important bioactive compounds from TCM formulae[31]. Our network pharmacology analysis explored bioactives, core therapeutic target genes, and potential mechanism of XQLD against CVA. Demonstrating functional potential of XQLD and its most active ingredients Mahuang and Guizhi in anti-inflammatory and anti-cytokine activity. Which was consistent with the previous study, which indicated XQLD reduced inflammatory cytokines and ER stress in COPD mice through downregulation of the AMPK/mTOR signalling pathway[11]. In addition, XQLD attenuated COPD in rats via inhibition of autophagy[12]. However, precise analysis becomes impossible considering the extremely large number of ingredient compounds (741) from the eight herbs in XQLD. By using the basic theory of TCM, we focus on the sovereign ingredients of XQLD, Mahuang and Guizhi. Our analysis of Mahuang-Guizhi revealed anti-inflammatory and anti-cytokines activities, which were consistent with the effects of XQLD in previous studies. Our results indicated Mahuang and Guizhi in XQLD play a major therapeutic role in the treatment of CVA. Moreover, a previous study demonstrated Mahuang decoction (another decoction including herb of Mahuang and Guizhi) mitigates airway inflammation and regulates the STAT3

signalling pathway[32]. Emphasizing the significance of Mahuang and Guizhi in inflammatory inhibition for CVA treatment. Meantime, the ingredients of Mahuang and Guizhi, Ephedrine, Pseudoephedrine, Anethole and Farnesol have been correlated to asthma therapeutic[33–36]. However, the CVA-specific active chemical compounds in Mahuang and Guizhi need further investigation. The 58 union target genes which we screened in the present study provided a basis for the next-step selection of the chemical compounds. Moreover, the therapeutic efficiency of the combination of XQLD and western medicines such as Montelukast is still unclear, and its mechanism needs further investigation.

IL-6/STAT3 is a key axis in autoimmunity, inflammation and cancer[37]. Peripheral blood IL-6 was recently identified as a potential biomarker in adult asthma[38]. Moreover, in contrast to adult asthma, children serum IL-6 levels were not associated with measures of disease severity or control such as symptoms and lung function. However, studies observed a significant association between IL-6 levels and risk of asthma exacerbations[39]. On the other hand, suppression of autophagy through JAK2/STAT3 contributes to the therapy of asthma, meanwhile serum IL-6 was downregulated[40]. Studies have shown that the transcription factor STAT3 plays an important role in the activation of the immune and structural cells, thus contributing to the development of asthma. Selective inhibition of STAT3 in animals significantly reduced the severity of lung inflammation[41]. Interestingly, our results demonstrated the significance of XQLD in regulating IL-6 and STAT3, via bioinformatics analysis through database and CVA mice model therapeutic experiment. Moreover, our analysis identified Interleukin family members IL1RN, IL2, IL2RA, IL4, IL5, IL-6, IL10, IL13, IL17A, and IL18R1 were included in the 58 union target genes of Mahaung and Guizhi for CVA treatment, indicating the importance of the interleukin family in the progress and treatment of asthma. Which was consistent with previous studies have reported interleukin family members IL4, IL5, and IL13 were involved in treatment of asthma[42]. However, the diagnostic limitations of this study should not to be ignored. Our data strengthen the theory of anti-inflammatory in immune response of XQLD in CVA treatment. In follow-up research, we will perform RNA-seq and transcriptomic analysis in the lung tissue of CVA mice following treatment with XQLD alone or in combination therapy with the first-line drug, Montelukast. It will further help in revealing the underlying molecular mechanism and genetic alteration following combinatorial therapy. This research may provide insight into developing a new therapeutic strategy for CVA by combing Chinese herbal medicine and Western medicine. The present study demonstrated the potential effect and the mechanism of XQLD in CVA treatment. Moreover, we proved that the active ingredients, Mahuang and Guizhi of XQLD have functional effects in regulating inflammation and cytokines secretion. Our findings suggest that XQLD effectively alleviated bronchial-lung tissue damage in CVA mice and inhibited the body inflammatory response by regulating IL-6/STAT3 signalling pathway.

Ethics approval and consent to participate

The research study was approved by the Ethics Committee of Human Experimentation at the Hospital. All animal experiments were carried out in accordance with the Experimental Animal Welfare Act and approved by Animal Experimentation Ethics Committee of our institution (Longgang District, Shenzhen, China, registered number: SZLDH2019LSYA-074, registered date: 2019.8.1).

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Author contribution

R.Y.: conceptualization, methodology, formal analysis, writing—review and editing. Z.Y.: conceptualization, methodology, formal analysis, writing—original draft. L.X.: software, validation, resources. Y.Z.: formal analysis, writing—review and editing.

Conflicts of interest disclosure

The authors have no conflicts of interest to declare.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Yan Zilong.

Data availability

The data described in this manuscript are contained in public databases or available from the corresponding author upon reasonable request.

Provenance and peer review

None.

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