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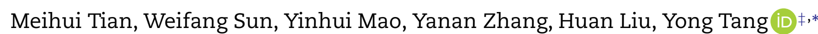
Case Study

Mechanistic study of acupuncture on the pterygopalatine ganglion to improve allergic rhinitis: analysis of multi-target effects based on bioinformatics/network topology strategie

Meihui Tian, Weifang Sun, Yinhui Mao, Yanan Zhang, Huan Liu, Yong Tang  ‡,*

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Abstract

One of the prevalent chronic inflammatory disorders of the nasal mucosa, allergic rhinitis (AR) has become more widespread in recent years. Acupuncture pterygopalatine ganglion (aPPG) is an emerging alternative therapy that is used to treat AR, but the molecular mechanisms underlying its anti-inflammatory effects are unclear. This work methodically demonstrated the multi-target mechanisms of aPPG in treating AR based on bioinformatics/topology using techniques including text mining, bioinformatics, and network topology, among others. A total of 16 active biomarkers and 108 protein targets related to aPPG treatment of AR were obtained. A total of 345 Gene Ontology terms related to aPPG of AR were identified, and 135 pathways were screened based on Kyoto Encyclopedia of Genes and Genomes analysis. Our study revealed for the first time the multi-targeted mechanism of action of aPPG in the treatment of AR. In animal experiments, aPPG ameliorated rhinitis symptoms in OVA-induced AR rats; decreased serum immunoglobulin E, OVA-sIgE, and substance P levels; elevated serum neuropeptide Y levels; and modulated serum Th1/Th2/Treg/Th17 cytokine expression by a mechanism that may be related to the inhibition of activation of the TLR4/NF-κB/NLRP3 signaling pathway. *In vivo* animal experiments once again validated the results of the bioinformatics analysis. This study revealed a possible multi-target mechanism of action between aPPG and AR, provided new insights into the potential pathogenesis of AR, and proved that aPPG was a promising complementary alternative therapy for the treatment of AR.

Keywords: acupuncture; pterygopalatine ganglion; allergic rhinitis; bioinformatics; network topology

Introduction

Recently, the prevalence of allergic rhinitis (AR) has been increasing yearly, with significant differences in prevalence from 5 to 50% globally [1]. Typical symptoms of AR are nasal leakage, nasal congestion, nasal itching, and sneezing [2]. AR not only seriously affects the quality of life of patients but also causes enormous socioeconomic burdens, as well as a risk of disability [3]. AR is a type I allergic reaction that occurs after the body is exposed to an allergen and is mediated by specific immunoglobulin E (IgE) [2]. AR is dominated by a type 2 inflammatory response, with imbalances in Th1/Th2/Th17 cytokines [4]. In particular, neurons, axonal reflexes, and neurotransmitters are also significant in the AR [5, 6]. AR is still not completely curable. Currently available medications or hormonal treatment regimens can improve the clinical symptoms of AR, but dry nasal mucosa, rhinorrhoea, insomnia, anxiety, and even central nervous system suppression and growth inhibition can also occur [1]. Therefore, exploring innovative therapies that are effective, safe, and cost-effective has become a new hotspot for research.

Acupuncture as a traditional medicine has been widely used by clinicians all over the world for a wide range of diseases [7]. Surprisingly, acupuncture is highly effective in treating allergic diseases characterised by type 2 inflammation [8]. Acupuncture

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of the acupuncture pterygopalatine ganglion (aPPG) is a promising complementary alternative therapy developed in recent years for the treatment of nasal mucosal inflammation [9]. A series of clinical randomised controlled studies and systematic reviews of aPPG for the treatment of AR are currently underway [10, 11]. The aPPG has been shown to be safe and effective in improving symptoms in patients with AR, with long-term efficacy as high as 70.4% [9]. In addition, it acts to improve nasal ventilation in healthy volunteers by regulating the autonomic nerve [12]. Functionally the pterygopalatine ganglion (PPG) controls vasoconstriction of the nasal respiratory mucosa, glandular secretion, and nasal sensation [13]. However, the therapeutic mechanism of aPPG for AR remains largely unclear.

It is worth mentioning that acupuncture exerts its therapeutic effect through a holistic regulatory effect on the organism, involving a network of multi-system, multi-level, and multi-target regulation [14]. With the advancement of research technology, acupuncture has also obtained a huge amount of clinical research data, and how to analyse and mine these data seems to be very necessary [15]. Bioinformatics/network topology has been widely used in pharmacological research, target prediction, and mechanistic studies [16]. In recent years, some scholars have applied bioinformatics to clearly predict the target of acupuncture treatment for COVID-19 and named this method as 'network acupuncture mechanism' [17]. This is of transgenerational significance for the traditional technique of acupuncture, because it breaks through the limitations of 'single target to single drug' research, and is more conducive to reflecting the law of action of acupuncture in treating diseases [18]. In the long run, bioinformatics can be used to predict the target effect of acupuncture in treating diseases, to guide clinical practice and to improve the efficiency of experimental research, thus reducing the waste of human, material, and financial resources [19].

In the present study, we aimed to reveal the multi-target action pattern of aPPG anti-AR by bioinformatics/network topology strategie, and further explore its regulatory mechanism by validating it with needling experiments in the AR rat model.

Methods

Collection of active biomarkers after aPPG treatment of AR

The following six databases were systematically searched in this study: Web of Science, Embase, PubMed, CNKI, vasoactive intestinal peptide (VIP), and Wanfang Databases (the latest update was 1 January 2024) for the following terms: acupuncture, electroacupuncture, PPG, pterygopalatine fossa, pterygopalatine region, pterygopalatine acupuncture point, xinwu acupoint, AR, rhinitis, rhinorrhea, sneezing, itchy nose, etc.

Eligible studies were as follows: (1) the aPPG treatment was considered to be stimulation using acupuncture targeting the PPG or pterygopalatine region. Excluding studies using other forms of stimulation such as acupressure point drug injections, acupressure without needles, radiofrequency thermo-coagulation, pulsed radiofrequency therapy, low-intensity focused ultrasound, neurectomy, and others. (2) The selected human clinical studies have clear efficacy indicators, including laboratory indicators of body fluids or serum. However, studies that evaluate efficacy solely through questionnaires and symptom scores are not included.

(3) Controlled interventions may have taken the form of another active treatment or medication. (4) Outcomes of interest were reported.

Literature search was completed by two researchers independently following pre-specified inclusion and exclusion criteria. Researchers read the full text and selected appropriate literature. Researcher disputes were resolved through negotiation. In the absence of agreement, a third researcher conducted the dispute resolution.

By searching the above databases, 16 active biomarkers were identified to have important associations with aPPG therapy for AR. Specifically, these are Interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, leukotriene D4, vascular cells adhere to molecules, nitric oxide, substance P (SP), VIP, neuropeptide Y (NPY), IgE, IgA, histamine [12, 20–29].

Bioinformatics/network topology analysis

Targets with high binding affinity for active biomarkers as described above were obtained from the STITCH database. The DisGeNET (http://www.disgenet.org/) and Genecards databases (http://www. genecards.org/) were used to identify the genes associated with AR. Overlapping targets of aPPG-associated targets with AR-associated targets were analyzed using the Venn tool(http://bioinfogp.cnb.csic.es/tools/venny), and the overlapping targets were considered as possible targets of aPPG for the treatment of AR. Protein–protein interaction (PPI) data were obtained from the STRING database (https://string-db.org) and nodes with a minimum correlation value higher than 0.4 were filtered. The generated file was loaded into Cytoscape 3.8.2 software to create PPI networks and Network Analyzer was used to determine the topological properties of the target network. Cytoscap 3.8.2 software was also used to construct composite target networks. The enrichment of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for core targets was examined using the Metascape (http://metascape.org/gp/index.htm l#/main/step1) database. The screening criteria were $P < 0.01$, and the results were visualized using the Bioinformatics website (https://www.bioinformatics.com.cn/).

Construction and evaluation of animal models with AR

Thirty healthy male Sprague Dawley rats (6–8 weeks old; specific pathogen-free grade) were adaptively fed under standard laboratory conditions for 7 days. Then, the rats were randomly divided into five groups (six rats per group): the control group (CON), AR model group (MOD), low-dose acupuncture group (AP-L), high-dose acupuncture group (AP-H), and budesonide nasal spray group (BUD). Except for the rats in the CON group, the rats in all the other groups were subjected to the procedures to establish AR. During the experiments in this study, the international principles of animal ethics were followed. The research protocol was approved by the Experimental Animal Ethics Committee of Changchun University of Chinese Medicine (approval number: 2023196). Figure 3(a) shows the specific experimental procedures of this study.

Rats in the MOD, AP-L, AP-H, and BUD groups were subjected to the protocol to establish AR, which consisted of three stages. To sensitize the rats in the first stage, 0.3 mg OVA (S12015-1 g, YUANYE, China) + 30 mg $Al(OH)_3$ (239186-25G, SIGMA, Germany) was prepared as a suspension using 1 mL of physiological saline as the solvent, and this suspension was intraperitoneally injected in the rats once every other day for a total of seven times. The second stage was the nasal cavity provocation stage: each rat was given nose drops of 5% OVA in physiological saline (50 μL per side) once a day for a total of seven times. In the maintenance stage, 50 μL of

5% OVA in physiological saline was sprayed into both sides of the nasal cavity of the rats once every other day for six consecutive days. Model evaluation: Ten minutes after the end of the last nasal drip in the nasal cavity provocation stage, the behavioral scores of the rats in each group were determined. The scale was as follows: (1) sneezing: 0—none; 1–1–3 times/10 min; 2–4–9 times/10 min; and 3— > 10 times/10 min; (2) nasal itching: 0—none; 1—rubbed nose 2 times/min; 2—rubbed nose 4–6 times/min; and 3—rubbed nose >6 times/min; (3) nasal discharge: 0—none; 1—discharge observed in a nostril; 2—discharge observed outside a nostril; and 3—discharge observed overflowed. The behavioral score was obtained by adding the various scores, and if the score exceeded 5 points, the model was considered to have been successfully established [30].

Acupuncture procedure

The rats in the AP-L group were needled once a day for 6 days. The rats in the AP-H group were needled twice a day (once in the morning and once in the evening, with a 6-hour interval between each needling on the same day) for a total of 6 days. Needling procedure: The operator pierced the skin at the upper edge of the zygomatic arch of each rat, and the needle tip pierced PPG in the pterygopalatine fossa toward the front, upward, and inward. The needle was removed after two to three strong stimulations, and pressure was applied to the site of the needle puncture with a sterilized dry cotton ball for 1 min. The schematic diagram of the needle puncture and the Micro CT (PerkinElmer, Inc. Waltham, MA, United States) 3D reconstruction model is shown in Fig. 3(b).

Budesonide nasal spray

The rats in the BUD group were treated with budesonide nasal spray (SE-15185, AstraZeneca AB, Sweden). A dose of 64 μg was delivered to each nostril twice a day for a total of 6 days.

Behavioral tests

The body weights of the rats were monitored on days 0, 14, 21, and 26, and all the rats were subjected to nasal symptom scoring on days 0, 21, and 26.

H&E, immunohistochemical, and immunofluorescence staining

After rat nasal mucosa samples were collected, they were fixed by paraffin embedding and then sectioned into 4-μm-thick sections. Partial sections were subjected to H&E staining, and the structural changes and inflammatory manifestations of the nasal mucosa were observed. Immunohistochemical staining was performed on some sections to assess the expression of IL-4 (1:200, Bioss, China) and IL-17 (1:200, Abcam, UK). Immunofluorescence staining was used to assess the expression levels of TLR4 (anti-TLR4, 1:200, Bioss, China) and MyD88 (anti-MyD88, 1:500, Proteintech, China). The stained tissues were observed under a confocal microscope imaging system (400x magnification, Nikon DS-U3, Tokyo, Japan). Quantification of average IHC/IF optical density (OD) values or relative fluorescence intensity values based on staining intensity was performed using Image-Pro-Plus 6.0 software (National Institutes of Health, Bethesda, MD).

Enzyme-linked immunosorbent assay

Sera and supernatants were collected and assayed using enzyme-linked immunosorbent assay (ELISA) kits to determine the rat IgE, OVA-sIgE, IL-6, IFN-γ, IL-10, TNF-α, SP, and NPY concentrations. The OD values were measured at 450 nm.

Table 1. The sequences of the primers for TLR4, MyD88, NLRP3, ASC, Caspase-1, IL-1β, IL-18, and β-actin.

Primer name	Primer sequence
TLR4 (FORWARD PRIMER)	GGGACTCTGATCATGGCATT
TLR4 (reverse primer)	GTCTCCACAGCCACCAGATT
MYD88 (forward primer)	AGGACAAACGCCGGAACTTTT
MYD88 (reverse primer)	CTGTTCTAGTTGCCGGATCATC
NLRP3 (forward primer)	TTTGTACCCAAGGCTGCTATCT
NLRP3 (reverse primer)	CACTCGTCATCTTCAGCAGCA
ASC (forward primer)	GTCTTAGGGGCGGAAACCAA
ASC (reverse primer)	CCGCGGTCACCTTTTACTCT
Caspase-1 (forward primer)	CCTGTCAGGGGCTCACTTTT
Caspase-1 (reverse primer)	TCCAAGTCACAAGACCAGGC
IL-1β (forward primer)	GCCACCTTTTGACAGTGATGAG
IL-1β (reverse primer)	GACAGCCCAGGTCAAAGGTT
IL-18 (forward primer)	CTGAATCCTGCCCCAGTGC
IL-18 (reverse primer)	CGGGGCCTGAGGATTATAGC
β-actin (forward primer)	TGAGCTGCGTTTTACACCCT
B-ACTIN (REVERSE PRIMER)	GCCTTCACCGTTCCAGTTTT

Western blotting assay

Protein blotting analysis was performed with anti-TLR4 (1:1000, PtmBio), anti-MyD88 (1:1000, PtmBio), anti-NLRP3 (1:1000, ABclonal), anti-ASC (1:1000, PtmBio), anti-Caspase-1 (1:1000, PtmBio), anti-IL-1β (1:1000, abcom), anti-IL-18 (1:1000, ZENBIO), anti-NF-κB-p65 (1:4000, Proteintech), anti-p-NF-κB-p65 (1:5000, Proteintech), and anti-β-actin (1:5000, Proteintech) antibodies. Rat nasal mucosal tissues were lysed with ice-cold RIPA buffer, and total proteins were separated by SDS–PAGE as previously described. After transfer, the membranes were blocked with 5% skim milk for 1 h and incubated overnight at 4 °C. The membranes were then visualized with a chemiluminescence detection system (ChemiDoc XRS, Bio-Rad). Western blot images were analyzed with ImageJ software.

Quantitative real-time PCR analysis

Total RNA was isolated from rat nasal mucosa tissues using a total RNA kit (Omega, Norcross, GA, USA) and then reverse transcribed into cDNA using a FastKing gDNA Dispelling RT SuperMix kit (KR118, Tiangen Biotech Co. Ltd., China). The qPCR analysis was performed with the Bio-Rad CFX96 System, and the relative mRNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method after normalization with the chamber control and the $2^{-\Delta\Delta Ct}$ method after normalization with the housekeeping control β-actin. The primer sequences are shown in Table 1.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). One-way ANOVA was used for comparisons between multiple groups, and the LSD-t test was used for two-way comparisons between groups. A $P < 0.05$ was considered to indicate a statistically significant difference. All the data were analyzed using GraphPad Prism 8.2.0 (GraphPad Software Inc., San Diego, California, USA).

Results

Target screening of aPPG in the treatment of AR

A flowchart showing the overall research strategy was shown in Fig. 1. Through literature mining 11 papers were included in this study and 16 active biomarkers of aPPG for the treatment of AR were screened (Table 2), and the application of the STITCH

Table 2. Summary of included trials and active biomarkers.

Study	Study design	Active biomarkers	Article type
Wang et al. 2016 [12]	RCT	SP, VIP, NPY, NO	Clinical study
Han et al. 2022 [20]	RCT	IL-4, IgE, IFN-γ	Clinical study
Yu et al. 2022 [21]	RCT	IL-4, IL-10, IFN-γ	Clinical study
Liu et al. 2022 [22]	RCT	IgE, SP	Clinical study
Yuan et al. 2020 [23]	RCT	IgA, IgE, IL-4, IL-5, IL-12, TNF-α	Clinical study
Liu et al. 2022 [24]	RCT	IL-4, IgE, IFN-γ, LTD4	Clinical study
Wu et al. 2022 [25]	RCT	IgE, sIgE,	Clinical study
Li et al. 2022 [26]	RCT	IL-4, IgE, IFN-γ	Clinical study
Li et al. 2022 [27]	RCT	IgE, SP	Clinical study
Li et al. 2020 [28]	RCT	VCAM-1, IL-4, IL-6, IL-10	Clinical study
Shao et al. 2020 [29]	RCT	IgE, histamine, IL-4	Clinical study

Table 3. Top 20 core targets.

Target name	Degree	Target name	Degree
IL1B	83	JAK2	60
IL6	82	NFKB1	59
TNF	82	PTPRC	59
IFNG	76	CSF2	57
IL4	76	IL5	56
IL10	74	ITGAM	56
STAT3	70	JAK1	55
IL13	68	EGFR	53
IL2	65	TP53	53
STAT1	60	CD40	53

database generated genes associated of active biomarkers. AR-related genes were collected from the DisGeNET and Genecards databases. Human targets were matched after normalizing gene names in the original file. Subsequently, 266 aPPG-associated targets and 2338 AR targets were utilized to draw a Venn diagram to get overlapping targets (Fig. 2A). In summary, our analysis showed that aPPG generated108 potential therapeutic targets of AR.

Analysis of PPI network and 'aPPG-Actives-Targets-Diseases' network

The PPI network was constructed based on the STRING database, and the topology is analyzed and then visualized by Cytoscape, showing 107 nodes and 1774 edges (Fig. 2B). The interaction between nodes is ranked by the size of the degree value.This study showed that IL-1β had the highest degree value (83), followed by IL-6 (82), TNF (82), IFNG (76), and IL-4 (76). The 20 core targets, ranked by degree value, were shown in Table 3, and these were identified as key targets for aPPG in the treatment of AR. The 'aPPG-Actives-Targets-Diseases' network to further investigate the mechanism of action of the important active biomarkers produced by aPPG to improve AR (Fig. 2C).

GO and KEGG enrichment analysis

The top 10 pathways with the most enriched GO analysis were shown in Fig. 2(D). Specifically, according to GO analysis, as far as cellular components are concerned core genes were predominantly expressed on structures such as side of membrane, receptor complex, and membrane raft, et al. At the biological process level, core genes are mainly involved in cell activation, cellular response to cytokine stimulus, and inflammatory response. In terms of molecular function, the core genes are mainly cytokine receptor binding, immune receptor activity, and IgE binding. KEGG pathway analysis indicated that aPPG may be involved in the regulation of signaling pathways such as the JAK-STAT signaling pathway, Neuroactive ligand-receptor interaction, Toxoplasmosis, PI3K-Akt signaling pathway, and AGE-RAGE signaling pathway in diabetic complications (Fig. 2E).

Improvement of rhinitis symptoms and body weight in rats with OVA-induced AR by aPPG

On the 21st day of the experiment, the rats in the MOD group, AP-L group, AP-H group, and BUD group showed obvious symptoms after OVA administration, and their AR symptom scores were higher than 5, which suggested that the model had been successfully established. On the 26th day of the experiment, compared with the MOD group, the rats in the AP-L and AP-H groups exhibited dose-dependent decreases in rhinitis symptom scores after needling the PPG via the pterygopalatine fossa ($P < 0.05$, $P < 0.0001$) (Fig. 3C). Interestingly, there was a significant increase in the body weight of the rats after high-frequency needling ($P < 0.05$) when compared with the MOD group (Fig. 3D).

The aPPG attenuated nasal mucosal pathology in rats with OVA-induced AR

H&E staining of rat nasal mucosal tissues showed that the nasal mucosal tissues of the MOD group were disorganized and obviously lacking cilia, with many more inflammatory cells infiltrating the submucosa than those of the CON group; the nasal mucosal tissues of the AP-L group did not show any significant improvement in structural disruption or poor layer organization, and they exhibited inflammatory cell infiltration; however, the nasal mucosal inflammation was improved in the AP-H and BUD groups, in which the infiltration of inflammatory cells was decreased compared with the MOD group, no obvious hematomas were observed, and the cilia were relatively well organized (Fig. 4A).

The aPPG corrected the imbalance of Th1/Th2/Treg/Th17 inflammatory factors and modulated autonomic function in AR rats

Immunohistochemical staining analysis (Fig. 4B) showed that the protein expression of IL-4, and IL-17 in the nasal mucosa of rats in the MOD group was significantly higher than that of rats in the CON group (all $P < 0.0001$), but aPPG reversed the overexpression of IL-4, and IL-17 that was observed in the MOD group in a dose-dependent manner (Fig. 4C–D). The results of ELISA showed that compared with the MOD group, the serum IL-6, TNF-α, IgE, and OVA-sIgE levels were decreased and the IL-10 and IFN-γ levels were increased after acupuncture (Fig. 4E–K). Additionally,

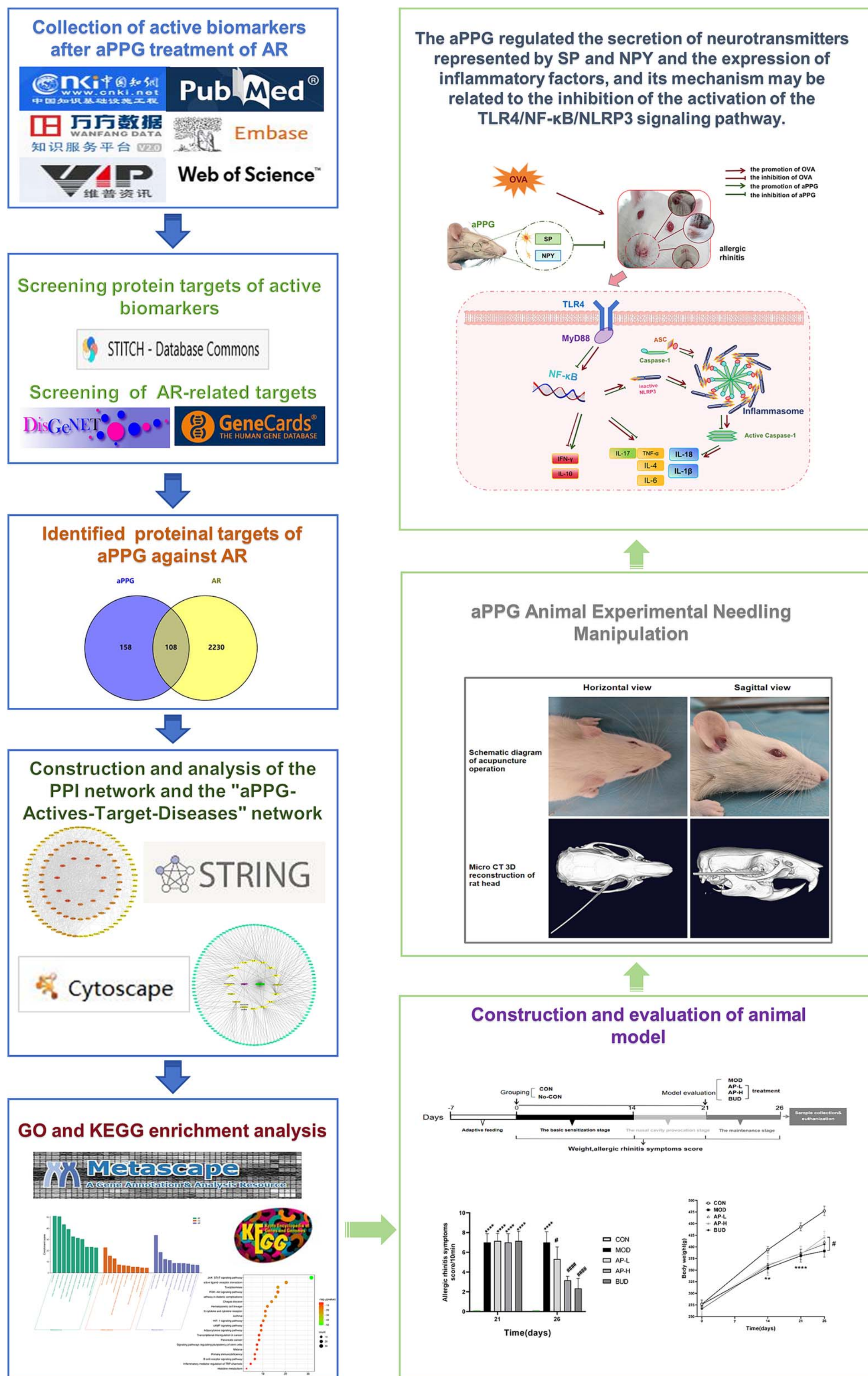


Figure 1. Flowchart of the overall research strategy.

compared with the MOD group, aPPG treatment decreased the serum SP levels in the AP-L and AP-H groups, and the NPY levels were increased (Fig. 5A–B). The results indicated that aPPG effectively modulated the balance of inflammatory factors and autonomic function through neurostimulation, further validated its neuroimmunomodulatory effects, which is consistent with the bioinformatics analysis.

The aPPG inhibited OVA-induced activation of the TLR4/NF-κB/NLRP3 pathway in rats with AR

To further determine the mechanism by which aPPG ameliorates AR, in this study, the co-expression of TLR4 and MyD88 in the nasal mucosa of rats was determined by dual immunofluorescence staining (Fig. 5E). The results showed that the relative fluorescence intensities of TLR4 and MyD88 were

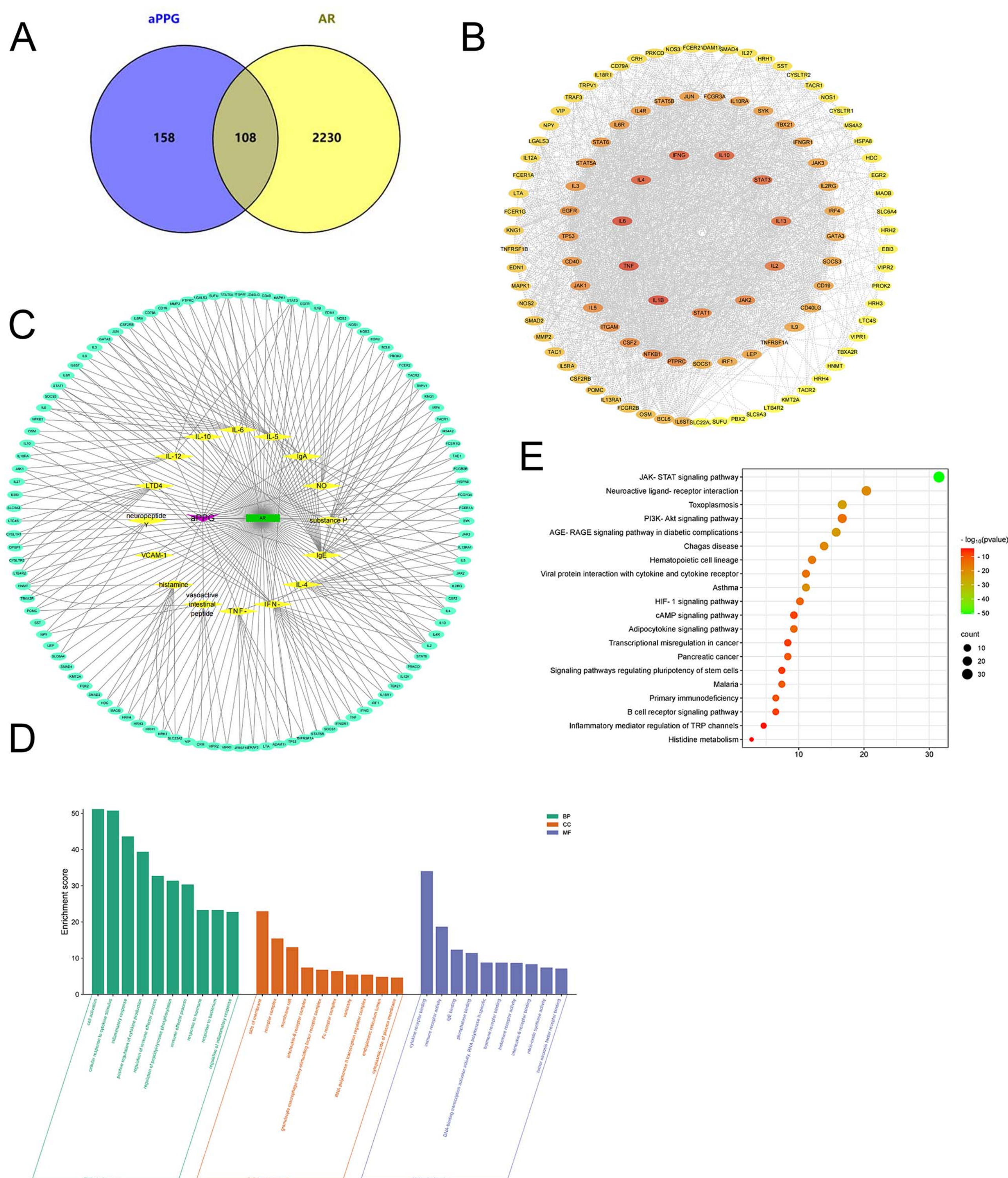
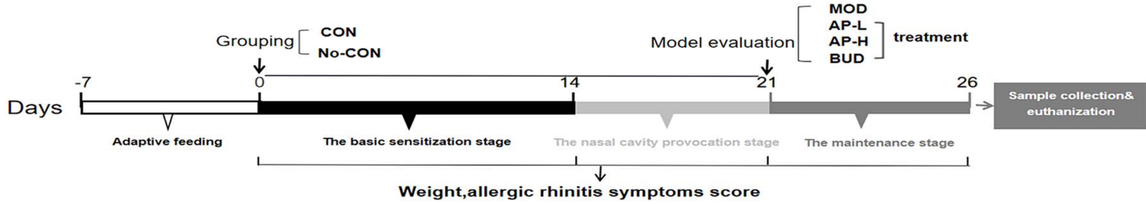
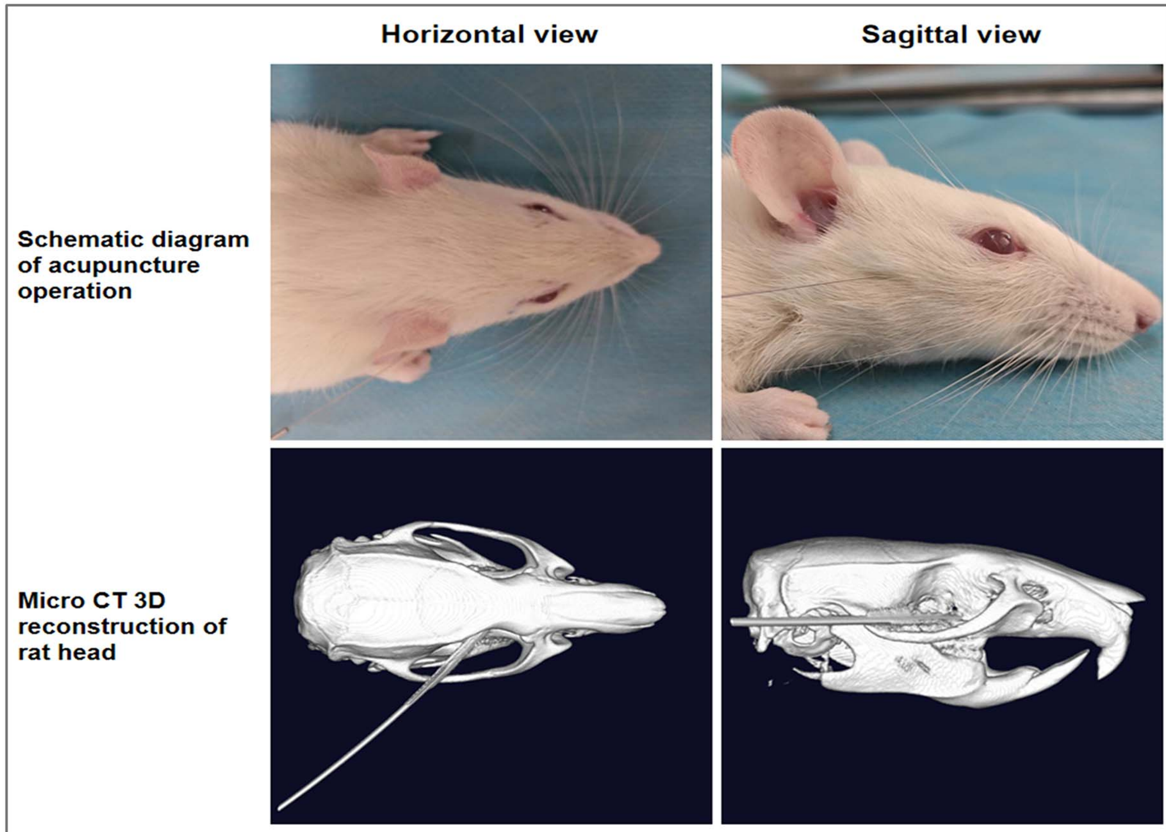
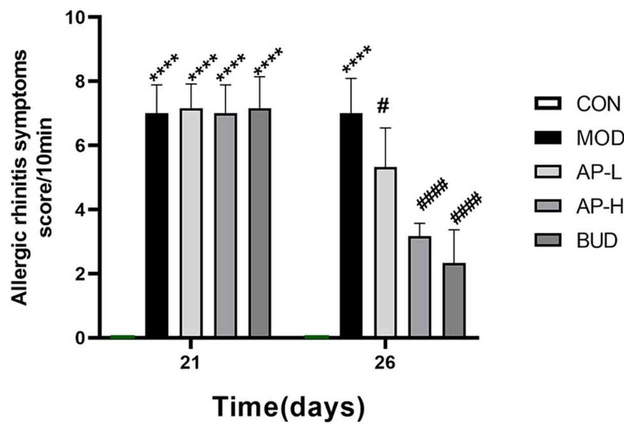


Figure 2. The potential mechanism of multi target and multi pathway effects of acupuncture pterygopalatine (aPPG) in treating AR. (A) Venn diagrams showed the number of the intersections generated between aPPG-associated targets and AR-associated genes. We identified 108 shared biotargets of aPPG against AR. (B) PPI network related to possible therapeutic targets of aPPG against AR. (C) Actives-targets-diseases network of aPPG for AR. (D, E) Employed GO and the KEGG enrichment analysis to explore the underlying mechanisms of aPPG against AR.

significantly higher in the MOD group than in the CON group (both $P < 0.0001$); the relative fluorescence intensity of TLR4 was lower in the AP-L group than in the MOD group ($P < 0.05$), and the relative fluorescence intensities of TLR4 and MyD88 were significantly lower in the AP-H group than in the MOD group (both $P < 0.001$) (Fig. 5C–D). The protein levels of TLR4, MyD88, p65, NLRP3, ASC, Caspase-1, IL-1β, and IL-18 in the nasal mucosa of rats in each group were measured by Western blotting (Fig. 6A). The results showed that aPPG suppressed TLR4, MyD88, NLRP3, ASC, Caspase-1, IL-1β, and IL-18 protein upregulation and inhibited the phosphorylation of NF-κBp65, and some of these changes occurred in a dose-dependent manner (Fig. 6B–I). To further elucidate the mechanism of action from the perspective of mRNA levels, the mRNA levels of TLR4, MyD88, NLRP3, ASC,







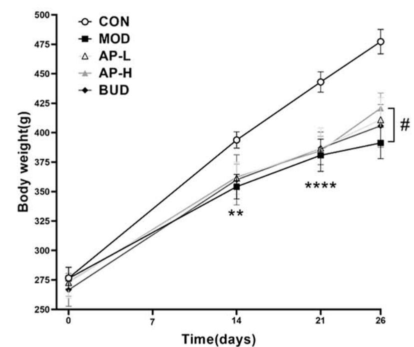


Figure 3. Modeling and acupuncture methods of allergic rhinitis rats. (A) Flowchart of the experimental design. (B) Schematic diagram of the needling method of aPPG. (C, D) Ameliorative effects of aPPG on rhinitis symptoms and body weight in rats. Mean ± SD, $n = 6$ rats per group. ∗∗$P < 0.01$, ∗∗∗∗$P < 0.0001$ versus CON group. #$P < 0.05$, ####$P < 0.0001$ versus MOD group.

Caspase-1, IL-1β, and IL-18 in the nasal mucosa of rats in each group were examined by quantitative real-time PCR. The results showed that aPPG decreased the mRNA levels of these factors compared with those observed in the MOD group (Fig. 6J–P).

Discussion

AR is a chronic inflammatory disease that affects the nasal mucosa and is associated with complex mechanisms [1]. The incidence of AR has gradually increased in recent years; AR is

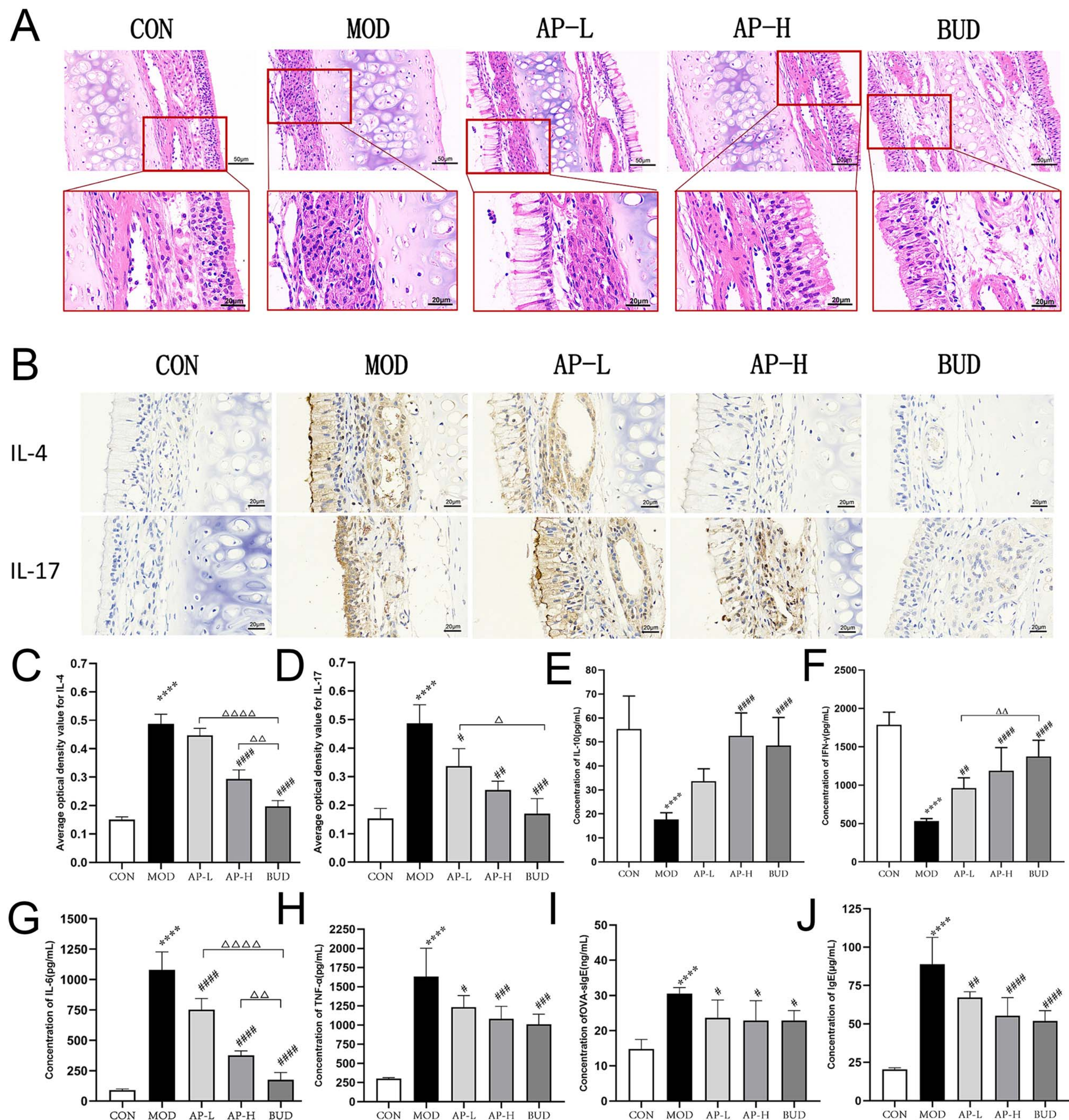


Figure 4. The aPPG-attenuated pathological damage and participated in the regulation of Th1/Th2/Treg/Th17 inflammatory factors in rat nasal mucosa. (A) H&E staining of rat tissues from each group (scale bar: 20 μm). (B–D) Immunohistochemical staining images from the various groups and their analytical results (scale bar: 20 μm). (E–H) Serum IL-10, IFN-, IL-6, TNF-α, OVA-sIgE, and IgE levels were measured by ELISA. Mean ± SD, $n = 6$ rats per group. ****$P < 0.0001$ versus CON group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, ####$P < 0.0001$ versus MOD group. $p < 0.05$, $p < 0.01$, $p < 0.0001$ versus BUD group.

characterized by recurrent episodes, and completely curing this disease is difficult [31]. aPPG is a novel, alternative treatment for AR. Previous studies have shown that aPPG improves nasal symptoms and quality of life, and it reduces the burden of daily illness in patients with AR; this treatment has the advantages of faster and longer duration of efficacy as well as shorter treatment duration, and the technique may also be effective in preventing the development of rhinitis [32, 33]. Although aPPG provides new ideas for AR treatment, its specific mechanism has not been reported.

Bioinformatics is an emerging field of systematically conducting mechanistic studies of acupuncture in an attempt to reveal the role of acupuncture in the treatment of specific diseases and interactions with multiple targets, a research methodology that analyses and exploits existing evidence, greatly improves laboratory efficiency, and is important for clinical decision making [18, 19]. In this context, the aim of the present study was to reveal the overall regulatory mechanism of aPPG on AR through a bioinformatics/network topology strategy. The results suggest that both gene targets of the nervous system and the immune-inflammatory system and their signalling pathways may be involved in the critical steps of aPPG in the treatment of AR.

In this study, the active biomarkers of aPPG against AR were collected by text mining, and we found that acupuncture can change the levels of allergic response mediators, neurotransmitters,

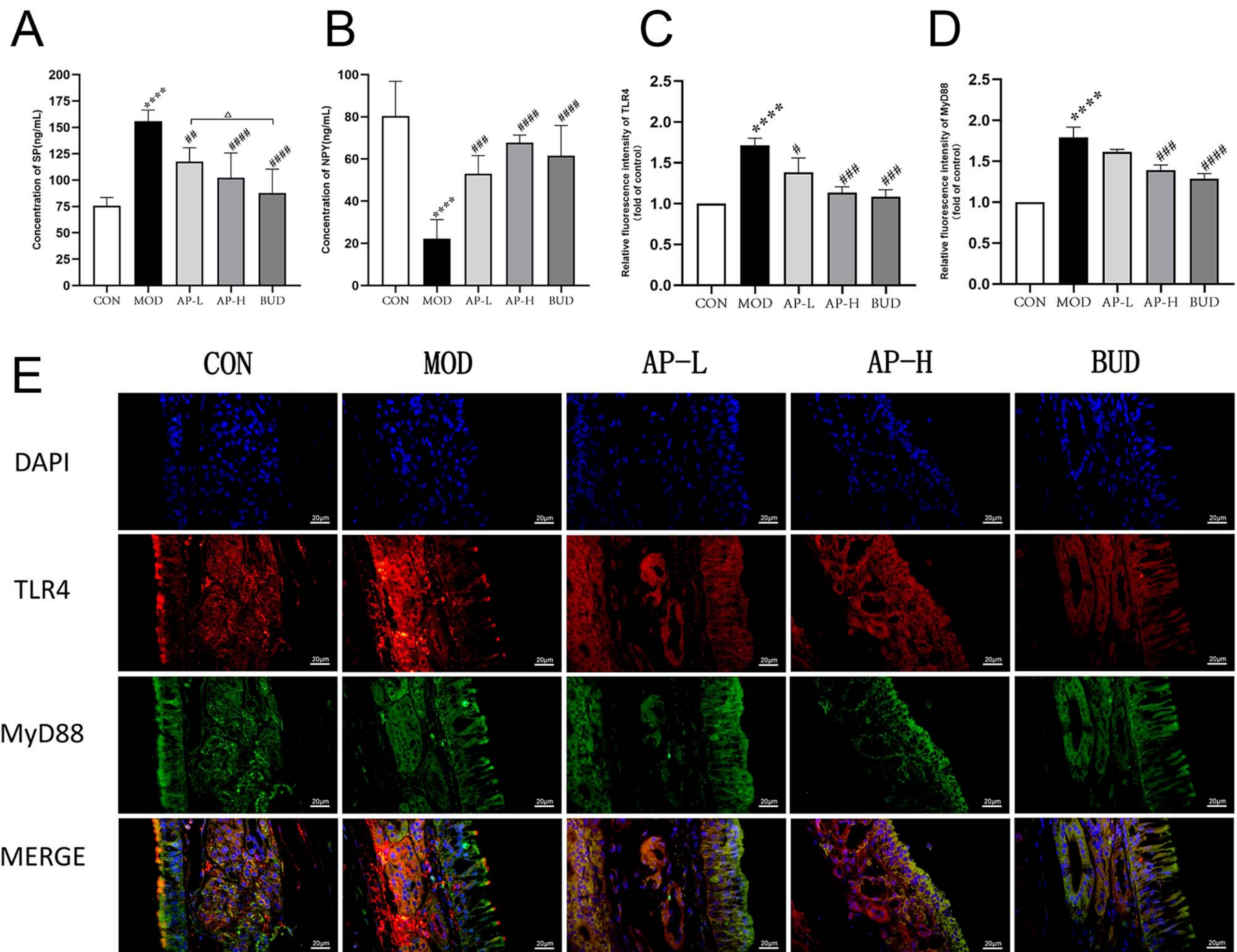


Figure 5. The aPPG regulated the levels of serum SP and NPY, as well as the expression of TLR4 and MyD88 in nasal mucosa. (A, B) Serum SP and NPY levels were measured by ELISA. (C–E) Immunofluorescence staining and analysis of TLR4 and MyD88 levels. Scale bar: 20 μm. Mean ± SD, $n = 6$ rats per group. ****$P < 0.0001$ versus CON group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, ####$P < 0.0001$ versus MOD group. $P < 0.05$ versus BUD group.

and various inflammatory cytokines in the body. When AR occurs, IgE cross-linking leads to mast cell activation, releases histamine, leukotrienes, promotes the secretion of cytokines such as IL-4, IL-5, and IL-6 by Th2 cells, and inhibits the expression and secretion of IFN-γ by Th1 cells [34]. TNF-α, a pro-inflammatory cytokine produced by mast cells, may be co-involved in the migration and activation of eosinophils, basophils, and Th2 cells [35]. Treg/Th17 cell imbalance has been shown to be strongly associated with inflammation in AR; Treg cell-derived IL-10 suppresses the immune-inflammatory response, whereas Th17 cells promote the inflammatory response [36]. In addition, levels of substance P (SP), a neurotransmitter released from sensory nerve endings that is involved in mast cell activation and promotes the release of inflammatory cytokines, have been found to be elevated in nasal secretions of patients with AR [37, 38]. Previous studies have shown that the development of AR is associated with parasympathetic hyperexcitability, and increased parasympathetic activity results in vasodilation of the nasal mucosa and increased secretions; conversely, sympathetic excitation promotes NPY expression, which can lead to nasal vasoconstriction and decreased secretion [39, 40].

In this study, A PPI network for aPPG for AR was established based on 108 overlapping genes of active biomarkers and disease genes. The top 20 targets ranked according to degree value were considered to have significant effects, including IL-1β, IL-6, TNF, IFNG, and IL-4. IL-1β has pro-inflammatory effects and is an important cytokine involved in and contributing to the development of AR, playing an important role in regulating the immune system in vitro and in vivo [41]. Meanwhile, IL-1β, a signature inflammatory factor secreted upon activation of NLRP3 inflammatory vesicles, plays a key role in the pathogenesis of AR [42]. This also inspired us to select the relevant pathways of NLRP3 inflammatory vesicles for subsequent validation in animal experiments. In addition, IL-1β belongs to JAK–STAT signalling pathway, Toxoplasmosis, and AGE-RAGE signalling pathway in diabetic complications, and KEGG pathway enrichment analysis showed that all of the above signalling pathways were highly enriched. Therefore, IL-1β may be an important target of aPPG for the treatment of AR. The high expression of IL-6 and IL-4 genes has a pro-inflammatory effect [43]; TNF has a regulatory effect on the body's immune function, which can act on T-cells, vascular endothelial cells, and has a significant enhancement of the biological effects of IL-1 [44]; the expression of IFNG (IFN-γ) has an enhanced immune function, and its expression is often inhibited in AR [4]. KEGG analysis showed that most of the enriched pathways were associated with inflammatory response, neuroactivity, and immunity. These pathways were mainly enriched in the JAK-STAT signaling pathway, Neuroactive ligand- receptor interaction,

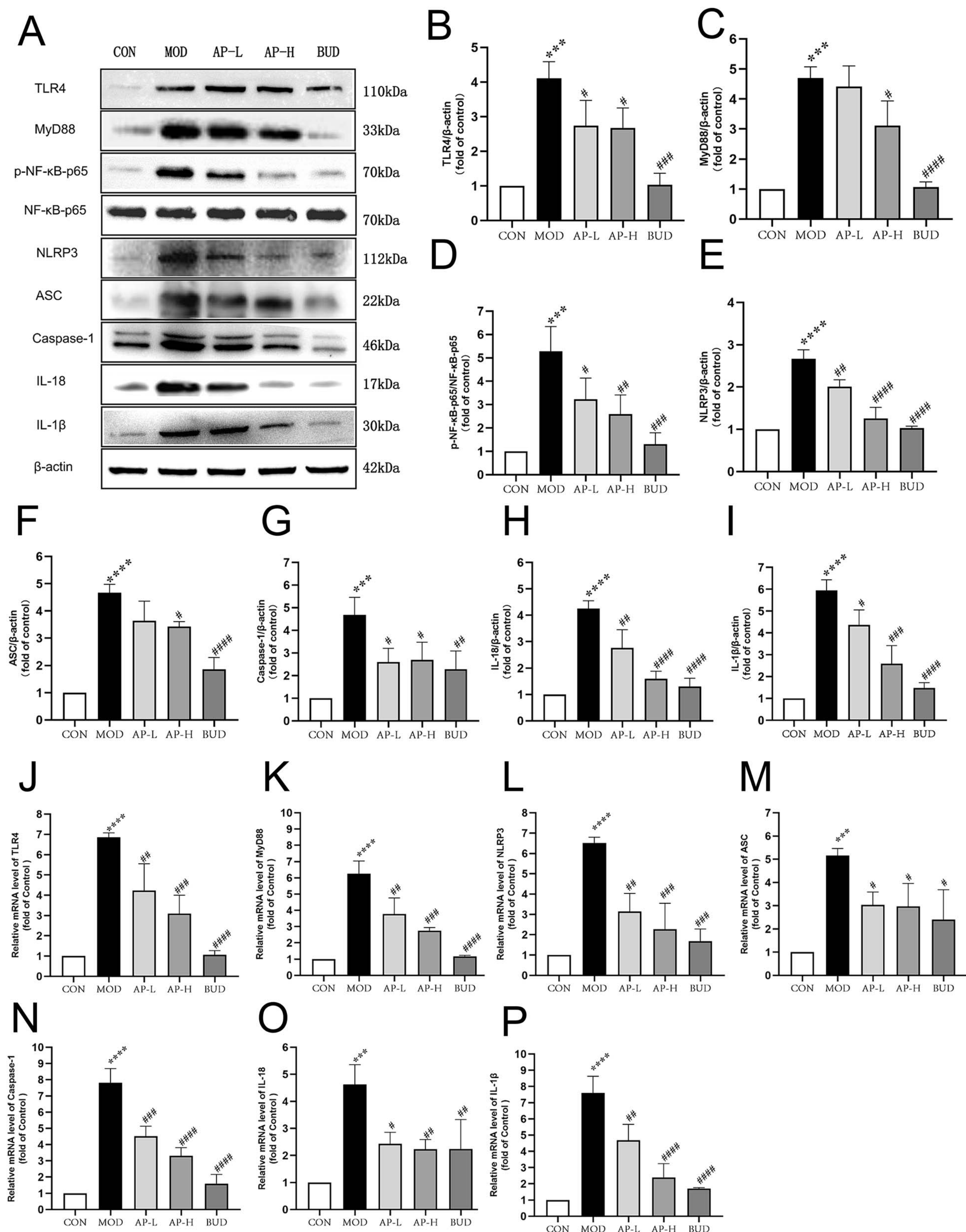


Figure 6. Effect of aPPG on the protein expression and mRNA expression of TLR4/NF-κB/NLRP3 signaling pathway. (A–I) The expression levels of TLR4, MyD88, p65, NLRP3, ASC, Caspase-1, IL-18, and IL-1β proteins in the nasal mucosa of rats in each group. (J–P) The mRNA expression levels of TLR4, MyD88, NLRP3, ASC, Caspase-1, IL-18, and IL-1β in the nasal mucosa of rats in each group. Mean ± SD, $n = 6$ rats per group. ***$P < 0.001$, ****$P < 0.0001$ versus CON group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, ####$P < 0.0001$ versus MOD group.

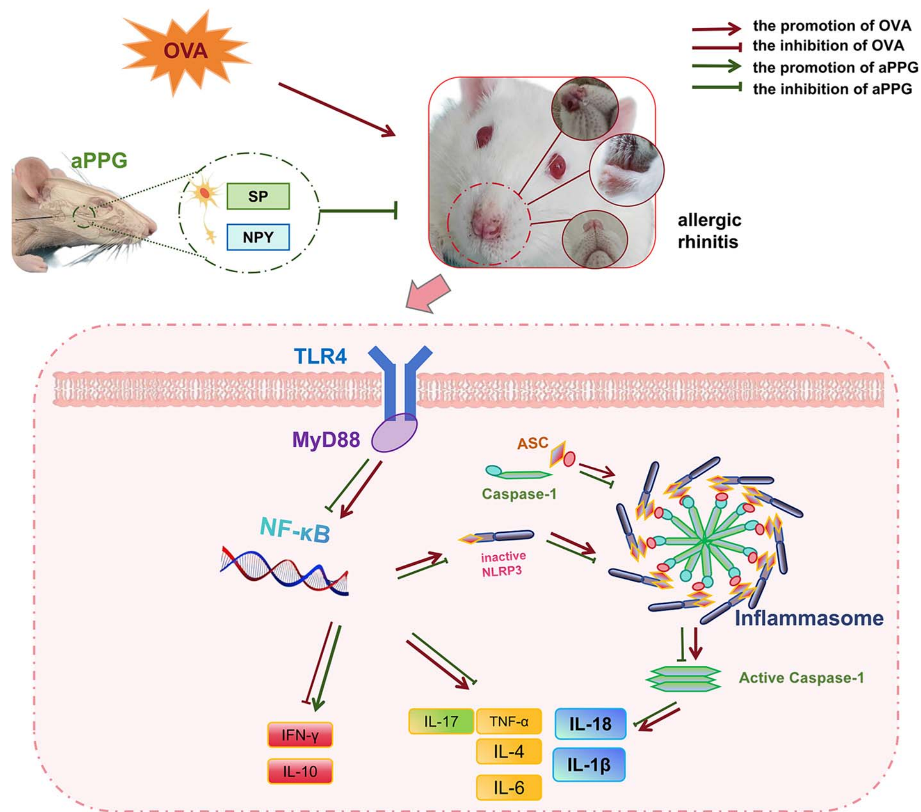


Figure 7. A working model of the mechanism of aPPG intervention in AR rats. The aPPG exerts an antiinflammatory effect, and its mechanism of action may be related to the fact that neurostimulation modulates the levels of neuromediators, represented by SP and NPY, and reduces inflammatory mediator expression by inhibiting the activation of the TLR4/NF-κ/NLRP3 signaling pathway.

and Toxoplasmosis. It has been found that the type 2 inflammatory response in AR can be inhibited by blocking the JAK–STAT signalling pathway [45]. Neuroactive ligand–receptor interaction and toxoplasmosis pathway are all neurologically related, suggesting that the activation or binding of the nervous system to the immune system can cause changes in neuronal function [46]. Overall, the neuro-immunomodulatory basis of aPPG for AR is inextricably linked to the modulation of the key targets and pathways described above.

To validate our findings, AR rats were modeled. Our observations centred around the bioinformatics prediction of key targets. The results showed that aPPG inhibited OVA-induced elevation of IgE, OVA-sIgE, TNF-α, IL-4, IL-6, and IL-17 levels and facilitated the secretion of IFN-γ and IL-10, suggesting that aPPG promotes Th1/Th2 and Treg/Th17 homeostasis. We also found that aPPG regulates the secretion levels of SP and NPY neurotransmitters associated with autonomic function, further validating that targets associated with neuroimmune inflammation are regulated by aPPG, which is consistent with previous bioinformatics target prediction results. In addition, we observed the NLRP3 signalling pathway with IL1β as a key downstream factor [47]. In recent years, studies have shown that aberrant activation of the NLRP3 inflammasome plays a key role in the pathogenesis of AR [48]. The NLRP3 inflammasome signaling pathway can be initiated by Toll-like receptors (TLRs); this initial step is followed by increases in cytokine production, activation of the nuclear factor-κB (NF-κB) signaling pathway to drive the transcription of NLRP3 receptor proteins and proinflammatory genes, activation of the adaptor protein ASC, formation of inflammasomes, and activation of Caspase-1, which promotes the release of several proinflammatory factors, including IL-1β and IL-18, and causes cellular pyroptosis [49, 50]. The release of inflammatory factors leads to the development of an amplified inflammatory cascade response, which ultimately promotes the onset and progression of AR [51]. Moreover, previous studies showed that NLRP3 inflammasomes were significantly overexpressed in the nasal mucosal epithelium of AR patients, and the exacerbation of AR may be related to NLRP3-mediated nasal mucosal epithelial cellular pyroptosis [48, 52]. In the present study, we focused on the TLR4/NF-κB/NLRP3 signaling pathway, and OVA-exposed rats showed high expression of TLR4, MyD88, NLRP3, ASC, Caspase-1, IL-18, and IL-1β, which promoted p65 phosphorylation. In contrast, aPPG inhibited the upregulation of these factors, exerting an excellent inhibitory effect on the TLR4/NF-κB/NLRP3 signaling pathway (Fig. 7).

AR has become a global health problem. The advantage of Chinese acupuncture lies in its overall network regulation, which is characterized by multi-level, multi-link, and multi target relationships and its network relationship requires bioinformatics prediction in order to promote clinical efficacy [18, 19]. It is worth noting that the standardization of aPPG manipulation in clinical practice may affect the therapeutic effect, and researchers should be rigorously trained in its operation [9]. Based on the results of our study, the targets of acupuncture PPG for AR treatment were systematically postulated and validated in animal experiments, and the neuro-immunological research mechanism of AR was thoroughly investigated, which is of far-reaching value in advancing the wide application of aPPG in clinical practice.

Limitations

Although the present study newly identified the multi-target action pattern of aPPG for AR, the number of high-quality clinical research studies on acupuncture is still in a scarce state [53], which may lead to the limitation of our findings. In this study, we explored the operation of needling the PPG in rats for the first time, and initially provided a simple validation of the results of the bioinformatics analysis, which still needs to be further carried out to enrich the experimental acupuncture in animals, to observe the neural-immunomodulation of the conduction loop of PPG by acupuncture, to scientifically explain the mechanism of acupuncture and to promote the improvement of clinical efficacy. In the future, we will carry out follow-up studies in this new direction.

Conclusions

In this study, we predicted for the first time the multi-target and multi-pathway mechanism of action of aPPG on AR using bioinformatics and network topology analysis. We found that acupuncture can alter the levels of allergic response mediators, neurotransmitters, and inflammatory cytokines *in vivo*. In addition, the aPPG significantly improved rhinitis symptoms in OVA-induced AR rats. The mechanism of action may be related to the fact that neural stimulation modulates the levels of neuromediators represented by SP and NPY and reduces the expression of inflammatory mediators by inhibiting the activation of the TLR4/NF-κB/NLRP3 signaling pathway. The present study suggests that aPPG is an adjunctive alternative therapy for AR with developmental potential.

Key Points

- For the first time, candidate targets and potential mechanisms for acupuncture pterygopalatine ganglion for the treatment of AR have been identified.
- This study helps advance mechanistic research on acupuncture pterygopalatine ganglion.
- This study proposes an acupuncture method for the pterygopalatine ganglion in rats.
- The present study suggests that acupuncture pterygopalatine ganglion is a complementary alternative therapy for AR with developmental potential.

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Ethical Statement

All animal experiments and protocols complied with international animal experimental ethics and requirements, and were approved by the Experimental Animal Ethics Committee of Changchun University of Chinese Medicine (Approval number: NO. 2023196).

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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