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RESEARCH ARTICLE

Preclinical and clinical studies on Qin-Zhu-Liang-Xue decoction: insights from network pharmacology and implications for atopic dermatitis treatment

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Abstract **To investigate the protective effects and underlying mechanisms of Qin-Zhu-Liang-Xue decoction (QZLX) in atopic dermatitis (AD) and glucocorticoid resistance, we conducted a single-blinded, randomized controlled clinical trial to evaluate the efficacy and safety of this concoction. Network pharmacology analysis was performed and validated through clinical studies. The efficacy, safety, and mechanism of action of QZLX and glucocorticoid receptor (GR) α recombinant protein were assessed in AD mice induced by 2,4-dinitrofluorobenzene (DNFB). Correlation analysis was performed to determine the clinical relevance of GRα. The trial demonstrated that patients who received QZLX showed considerable improvements in their Scoring Atopic Dermatitis (SCORAD) and Dermatology Life Quality Index (DLQI) scores compared with those who received mizolastine at week 4. Network pharmacological analysis identified GRα as a key target for QZLX in AD treatment. QZLX administration increased the serum GRα expression in AD patients, alleviated AD symptoms in mice, decreased inflammatory cytokine expression, and increased GRα expression without affecting liver or kidney function. In addition, GRα recombinant protein improved AD-like skin lesions in DNFB-induced mice. A negative correlation was observed between GRα expression and clinical parameters, including SCORAD, DLQI, and serum IgE levels. QZLX alleviates AD symptoms through the upregulation of GRα and thus presents a novel therapeutic strategy for the prevention of glucocorticoid resistance in AD management.**

Keywords Qin-Zhu-Liang-Xue decoction; atopic dermatitis; glucocorticoid receptor α; traditional Chinese medicine; network pharmacology

Introduction

Atopic dermatitis (AD) refer to a prevalent chronic and recurrent inflammatory skin disease characterized by pleomorphic skin lesions, dry skin, and intense itching [1]. AD among children has a prevalence in the range of 2.7% to 20.1%, and the value ranges from 2.1% to 4.9% in adults across different populations [2,3]. The pathophysiology of AD is multifaceted and involves genetic and environmental factors, which present tremendous treatment challenges [4].

The current treatment options for AD include topical glucocorticoids, topical calcineurin inhibitors, systemic immunosuppressants, and biologic agents [5]. Despite being the mainstay of AD treatment, topical glucocorticoids face limitations due to safety concerns and reduced efficacy with prolonged use [5]. Long-term or excessive use of glucocorticoids has been linked to adverse effects such as skin atrophy [6]. Moreover, glucocorticoid resistance in AD patients substantially diminishes the effectiveness of treatments. Glucocorticoids exert immunomodulatory and anti-inflammatory

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effects by binding to their receptor, the glucocorticoid receptor (GR) [7,8]. Glucocorticoid resistance in AD patients is linked to the reduced expression of GRα in peripheral blood mononuclear cells [9]. The low expression and decreased binding affinity of GR contribute to AD development and severity [10–12]. Therefore, the achievement of a balance that maximizes the beneficial anti-inflammatory actions of glucocorticoids while ensuring their safe use is crucial in AD treatment.

Traditional Chinese medicine (TCM) has been used for centuries as an integral component of complementary and alternative medicine used in the treatment of skin diseases [13]. Qin-Zhu-Liang-Xue (QZLX) decoction demonstrates a remarkable efficacy in the clinical treatment of AD, with a response rate of approximately 86.7% when used in conjunction with moisturizers [14]. This decoction also exhibited a low recurrence rate of 6.7% during a 4-week follow-up period [15]. The QZLX decoction consists of 10 herbal and mineral medicines (Table 1). Baicalin and glycyrrhizic acid are the primary active ingredients of QZLX decoction [15,16]. Baicalin ameliorates AD through the suppression of skin inflammation and improvement of skin barrier function [17]. Glycyrrhizic acid alleviates AD-like symptoms by suppressing Th1/Th2/Th17 immune responses [18]. However, further systematic research is required to elucidate the mechanism of QZLX in the treatment of AD due to the complex nature of TCM, which involves multiple components and targets.

Network pharmacology, which is rooted in systems biology, polypharmacology, and molecular networks, is extensively used in the analysis of the interactions among drugs, components, diseases, and targets [19]. This approach aligns with the holistic philosophy of TCM and is widely employed in the investigation of its intricate pharmacological mechanisms [20].

In this study, we identified GR as the central target of QZLX via network pharmacology. We subsequently explored the pharmacological effects of QZLX on AD and investigated its molecular mechanisms associated with GR through clinical trials and animal experiments. Our findings illuminate the clinical relevance of GRα in AD and propose an effective strategy for the enhancement of GRα expression and prevention of glucocorticoid resistance in AD treatment.

Materials and methods

Drug administration and quality control

The QZLX decoction, which comprises ten Chinese herbal and mineral medicines (Table 1), was used in this study. The dosage followed the guidelines provided by the Chinese Pharmacopoeia (2015 edition). The QZLX decoction, which was stored at 4 °C, was manufactured by Shanghai Baolong Pharmaceutical Co. LTD (Z20200018000) and supplied by the pharmacy department of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine. Our previous research identified baicalin and glycyrrhizic acid as primary components of the QZLX decoction. High-performance liquid chromatography (HPLC) methods were performed as previously described to ensure the quality of QZLX [15,16].

Clinical study design

This work involved the conduct of a prospective, two-arm, single-blinded, randomized trial at Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, affiliated with Shanghai University of Traditional Chinese Medicine in Shanghai, China, from June 1, 2020, to December 31, 2021. The study protocol and consent forms were approved by the Institutional Review Board of Yueyang Hospital (No. KYSKSB2020-125) and registered in the Chinese Clinical Trial Registry

Table 1 Ingredients and dosage of QZLX decoction (per 1000 mL)

Chinese name	Latin name	English name	Dosage (g)
Huang Qin	*Scutellaria baicalensis* Georgi	Radix Scutellariae	166
Zhen Zhu Mu	Concha Margaritifera Usta	Nacre	500
Zi Cao	*Arnebia endochroma* Aitch.	Radix Arnebiae	250
Ling Ci Shi	Magnetitum	Magnetite	500
Sheng Mu Li	*Crassostrea gigas*	Oyster Shell	500
Yi Yi Ren	*Coix lacryma-jobi* L.	Jobstears Seed	500
Fang Feng	*Saposhnikovia divaricate* (Turcz.) Schischk	Radix Sapoahnikoviae	166
Xu Chang Qin	*Cynanchum paniculatum* (Bge.) Kitag	Radix Cynanchi Paniculati	333
Fo Er Cao	*Gnaphalium affine* d'Urv.	Cudweed	166
Gan Cao	*Glycyrrhiza uralensis* Fisch.	Radix Glycyrrhizae	100

(ChiCTR2000037034). In addition, this work was performed in compliance with the principles of the Declaration of Helsinki and Tokyo for human subjects, and a written informed consent was provided by all participants before enrollment. A total of 131 patients were randomly assigned to either the control or treatment group via cluster randomization. The treatment group received oral administration of 30 mL QZLX decoction, twice daily for 4 weeks, and the control group received a 10 mg oral mizolastine sustained-release tablet (Minzhilin, Xi'an-Janssen Pharmaceutical Co., Ltd.), each night for 4 weeks. Both groups received basic topical treatment, including moisturizer and 0.05% desonide cream (Liyanzhuo, Chongqing Huabang Pharmaceutical Co., Ltd.). The Scoring Atopic Dermatitis (SCORAD) [21] and Dermatology Life Quality Index (DLQI) [22] were used as outcome measures and assessed at baseline and weeks 2 and 4.

Sample collection

A 15 mL blood sample was collected from each patient prior to and at the end of the trial for study and safety evaluations, including the measurements of serum total IgE, serum GRα, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen (BUN), and serum creatinine (Scr). Skin samples were obtained from patients with AD and healthy volunteers (n = 4) who provided written informed consents. A 4 mm skin punch biopsy was collected from skin lesions of the AD patients and lower back skin of normal controls. Skin biopsies were treated with a 4% formalin solution before histological examination.

Network pharmacological analysis

Network pharmacological analysis was conducted following a previously described methodology [23]. Briefly, HIT 2.0 database, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine, and LTM-TCM database were used to search for active compounds present in the QZLX formula. The corresponding targets of these compounds were retrieved from the aforementioned databases and Swiss Target Prediction database. To compile the disease-related targets, we utilized the Therapeutic Target Database, OMIM database, and GeneCards database. Protein–protein interaction (PPI) data were obtained from STRING database. Network visualization was conducted using Cytoscape software, with its CytoNCA plug-in used in parameter analysis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the DAVID database, and the results were visualized using R language.

Animals experiment

Animals

A total of 40 male C57BL/6 mice (8 weeks old) were purchased from Shanghai Slac Laboratory Animal Company. The mice were housed in a sterile and specific pathogen-free facility with standard temperature regulation (23 ± 2 °C) and provided ad libitum access to standard food and water. The animal experiments received approval from the Experimental Animal Ethics Committee of Yueyang Hospital (YYLAC-2019-014-1; YYLAC-2019-064-6) and adhered to ethical standards.

Model establishment and grouping

The AD-like mouse model was established in accordance with a previously published protocol [24]. 2,4-Dinitrofluorobenzene (DNFB, Sigma, St. Louis, MO, USA) was dissolved in acetone and olive oil (4:1 in volume). On day 0, a 2.0 × 3.0 cm^2 area of the mice's back hair was shaved. On day 1, except for the control group, all mice were topically treated with 50 μL 0.5% DNFB solution. On days 5, 8, 11, and 14, a total of 20 and 50 μL 0.2% DNFB were applied to the right ear and the back, respectively, excluding those of the control group. The control group received acetone and olive oil (4:1) without DNFB. Orally administration of the QZLX decoction to the mice was achieved via daily gavage at three different dosages: 1.3 (QZLX-L), 1.9 (QZLX-M), and 2.5 g/kg (QZLX-H), over 16 days. Based on the findings displayed in Fig. S1, in subsequent experiments, an optimal concentration of 1.9 g/kg was used in the DNFB + QZLX group. The DNFB + GRα group received daily intraperitoneal injection of recombinant GRα protein (ZY774Mu011, HZbscience, Shanghai, China) at a dose of 0.02 mg/kg for 16 days. The control and DNFB groups received purified water via oral gavage throughout the experiment. On day 16, all mice were euthanized after the assessment of back-skin lesions and collection of blood samples.

Clinical symptoms and scratching assessment

The regional Eczema Area and Severity Index (EASI) scores of AD-like lesions in mice were evaluated before and after treatment [25]. Scores ranging from 0 (none) to 4 (severe) were assigned for erythema/hemorrhage, scaling/dryness, edema/exudation, and abrasion/erosion. Mice were given a 30 min adaptation period in their cages before recording and quantification of their scratching behavior. Each instance of hind paw movement toward the itching area followed by its return to the ground was

considered a scratching event. An investigator blinded to the grouping performed the assessments.

Histological evaluation

Skin lesion samples were fixed in 10% formaldehyde, embedded in paraffin, and subjected to standard hematoxylin and eosin (HE) staining. Histological alterations were examined using a light microscope, with a focus on epidermal hyperplasia, spinous layer hypertrophy, tissue edema, and inflammatory cell infiltration in four randomly selected fields under 40× magnification.

Immunohistochemistry (IHC)

IHC was conducted in accordance with established protocols [26,27]. The primary antibodies used included the following: anti-GRα antibody (1:1200, ab3580, Abcam), anti-GRβ antibody (1:1800, ab233165, Abcam), anti-nuclear factor kappa B p105 (NF-κB p105) antibody (1:60000, ab32360, Abcam), anti-CD4 antibody (1:300, ab237722, Abcam), and anti-human CD4 antibody (1:300, ab133616, Abcam). Epidermal thickness and positive cell rates were quantified following previously described methods [16,26].

Enzyme-linked immunosorbent assay (ELISA)

The expression levels of GRα, IgE, interleukin (IL)-4, tumor necrosis factor-α (TNF-α), IL-22, and IL-17 were determined using the respective ELISA kits for the following: human GRα (EK-H11655, EK-Bioscience), mouse GRα (EM1092, Li Rui Biological Technology), mouse IgE (ab157718, Abcam), mouse IL-4 (ab100710, Abcam), mouse TNF-α (ab208348, Abcam), mouse IL-22 (ab223857, Abcam), and mouse IL-17 (ab157718, Abcam). Specific ELISA kits were also used for the measurement of the serum levels of ALT (ab282882, Abcam), AST (ab263882, Abcam), Scr (E-EL-M1228, Elabscience), and BUN (YS02947B, GTX). All ELISA procedures followed the manufacturer's instructions.

Reverse transcription–polymerase chain reactions

Total RNAs were isolated from mouse skin tissues using Trizol reagent and reverse transcribed into cDNA templates. Amplification of the target genes was performed using the following specific primers (5′–3′): GRα (forward GTGTATTATTGGCAACCTATGAG and reverse CCCAGTGAGATTACAGAGGAAGT) and GAPDH (forward GCACCGTCAAGGCTGAGAAC and reverse ATGGTGGTGAAGACGCCAGT). Relative gene expressions were quantified using Syber Green and calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Data analysis was conducted using SPSS 25.0. Categorical variables were presented as numbers and percentages and continuous variables as means and standard deviations (mean ± SD). Multiple group comparisons were performed using one-way analysis of variance, followed by Tukey's post-hoc test. Student's *t*-test was employed for comparisons between two groups. Spearman's rank correlation was used to calculate correlations. Statistical significance was considered at $P < 0.05$.

Results

QZLX decoction ameliorated skin lesions in AD patients

This study initially included 131 participants (Fig. 1). Among the participants, 14 dropped out, which left 117 patients who completed the prescribed treatment course. Table 2 summarizes the baseline characteristics of AD patients. No statistically significant differences were observed between the groups in terms of age, sex, duration, or severity of AD.

Following treatment with QZLX, AD patients exhibited considerable improvement in their skin lesions (Fig. 2A). Specifically, the SCORAD score decreased at weeks 2 and 4 in both groups, with the QZLX group exhibiting a significantly greater improvement compared with the control group ($P < 0.001$) (Fig. 2B). At week 4, the QZLX group presented a significantly lower DLQI score than the control group ($P < 0.05$) (Fig. 2C). Although serum IgE levels decreased in both groups at week 4, no significant difference was detected between them (Fig. 2D). Importantly, no serious adverse events were reported during the study period, and QZLX showed no significant influence on the serum levels of ALT, AST, BUN, or Scr (Fig. 2E–2H). These findings demonstrate the effective and safe use pf QZLX in AD treatment.

Network pharmacological analysis identified GRα as a hub target of QZLX in AD treatment

To elucidate the mechanism of QZLX in AD treatment, we employed network pharmacology and conducted clinical validation. We initially screened 609 active ingredients in QZLX, along with their 1151 targets, and obtained 1980 AD-related genes from databases. From this step, we determined 361 overlapping genes between the target genes of active ingredients and AD-related genes, which were predicted as candidate targets of QZLX for AD treatment (Fig. 3A).

Subsequently, we constructed a compound-target network of QZLX and filtered it based on degree scores above the average value. As a result, a subnetwork

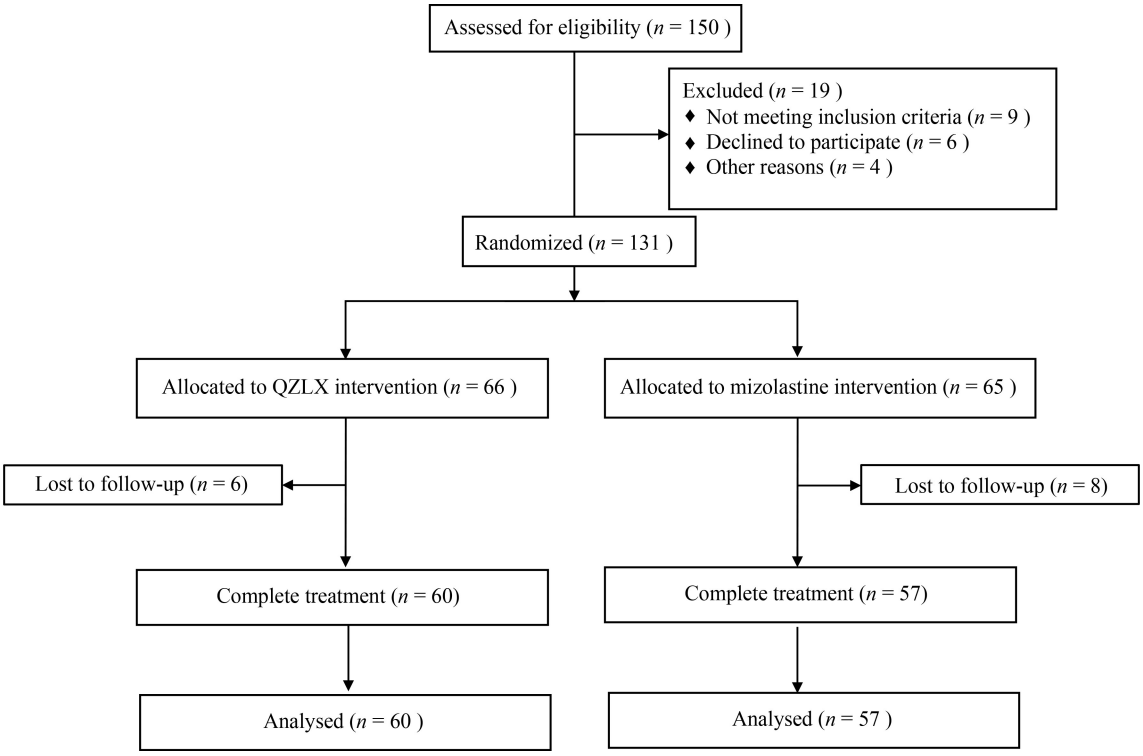

Fig. 1 Patient flow chart.

Table 2 Baseline demographic and clinical characteristics of patients included in the study

Characteristic	QZLX (n = 60)	Control (n = 57)	P value
Age, year	44.1 (9.5)	42.0 (8.7)	0.214
Sex (n(%))			0.242
Female	21 (35)	26 (46)	
Male	39 (65)	31 (54)	
Duration of AD, m	11.2 (10.2)	10.6 (9.5)	0.553
SCORAD score	45.7 (4.9)	46.2 (4.8)	0.542
DLQI score	16.1 (3.1)	16.9 (3.1)	0.182
IgE, IU/mL	585.3 (261.3)	696.0 (236.4)	0.168

Data are displayed as mean (SD) unless otherwise indicated. AD, atopic dermatitis; SCORAD, Scoring Atopic Dermatitis; DLQI, Dermatology Life Quality Index.

consisting of 159 nodes and 1055 edges, encompassing 74 candidate targets and 85 ingredients, was obtained (Fig. 3B). To further gain insights into candidate targets, we performed KEGG and GO enrichment analyses using DAVID. GO analysis revealed enriched terms, including response to drug, aging, cellular response to cadmium ion, response to lipopolysaccharide, inflammatory response, response to estradiol, and intracellular steroid hormone receptor signaling pathway (Fig. 3C).

PPI networks were also constructed using the STRING database with the 74 candidate targets, and topological analysis was performed to identify core genes based on comprehensive ranking criteria, such as degree, betweenness, and closeness. As a result, we identified seven core genes: *NFKBIA*, *HSP90AA1*, *TNF*, *AKT1*, *IL6*, *NR3C1*, and *MMP9* (Fig. 3D). Based on the findings of GO enrichment analysis and the presence of core genes, we hypothesized that the glucocorticoid receptor, which is encoded by *NR3C1*, is a crucial target of QZLX. To validate this hypothesis, we conducted ELISA to assess the expression levels of GRα in the clinical serum samples. Consistent with our hypothesis, the expression level of GRα was upregulated after QZLX treatment ($P < 0.001$), whereas no significant difference was observed in the control group before and after treatment ($P > 0.05$) (Fig. 3E).

QZLX attenuated DNFB-induced AD-like dermatitis in mice

We further validated our initial findings through animal experiments using a mouse model of DNFB-induced AD-like dermatitis. Consistent with previous reports, baicalin and glycyrrhizic acid served as standard samples for quality control, and HPLC analysis was performed to assess the quality of QZLX (Fig. S2). After 2 weeks of treatment, the severity of skin lesions in the AD mice was significantly alleviated (Fig. 4B). In addition, the frequency of scratching behavior and the regional EASI scores decreased markedly (Fig. 4C and 4D). Histological examination revealed that QZLX substantially improved

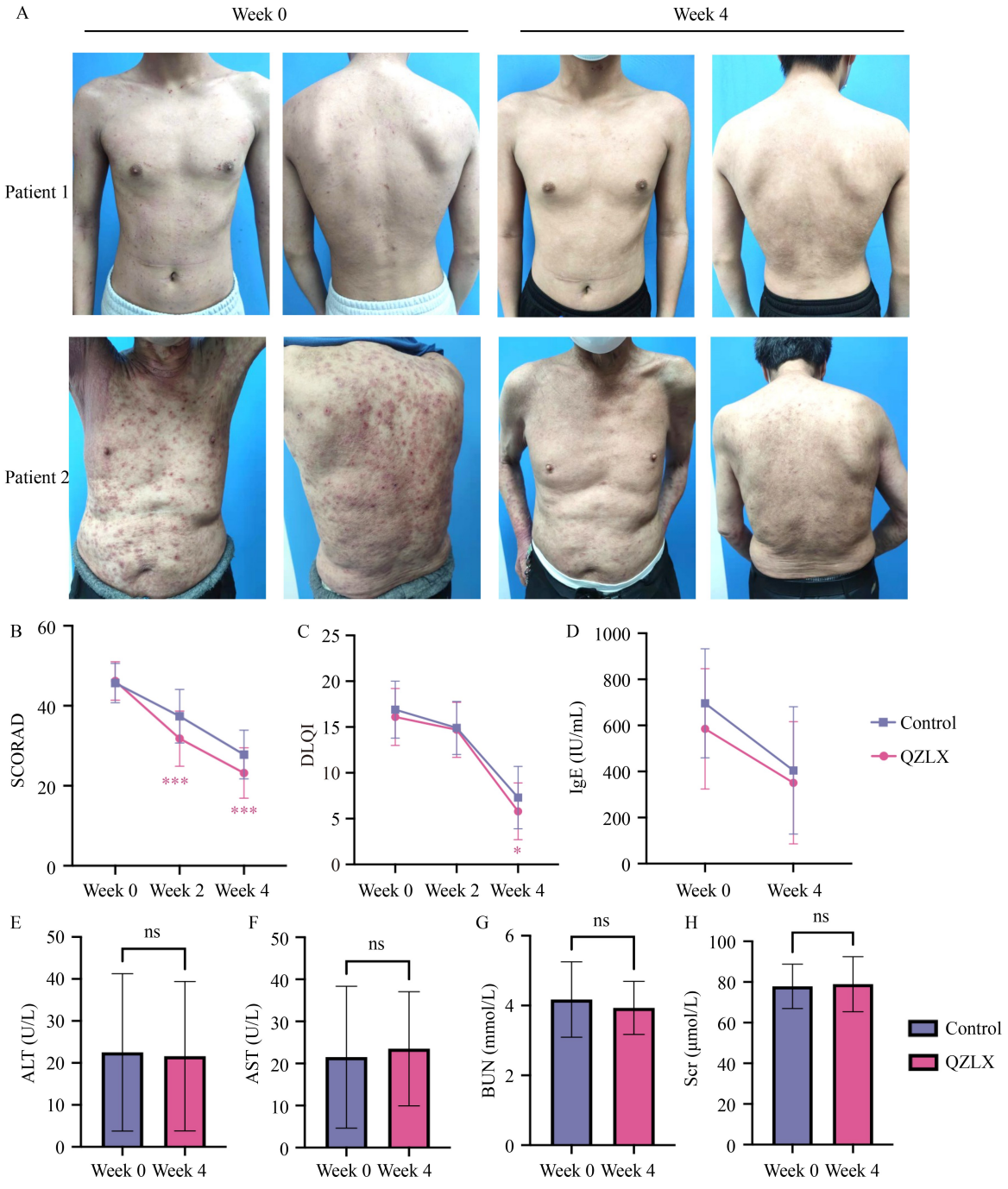

Fig. 2 QZLX treatment alleviated AD symptoms in patients. (A) Representative photographs of regional lesions in AD patients at weeks 0 and 4 after QZLX treatment. Changes in the SCORAD index (B) and DLQI (C) at baseline (week 0), week 2, and at the end of the treatment phase (week 4). (D) Serum total IgE at baseline (week 0) and week 4. Safety assessments focused on the serum levels of ALT (E), AST (F), BUN (G), and Scr (H) before and after QZLX treatment. Data are presented as mean ± SD, $^{*}P < 0.05$, $^{***}P < 0.001$, compared with the control group.

pathological conditions, with notable reductions observed in the epidermal thickness and inflammatory cell infiltration in the back and ear skin lesions (Fig. 4E–4I). Furthermore, comparable serum levels of AST, ALT, Scr, and BUN were noticed between the DNFB and QZLX-treated groups (Fig. S3), which indicates the safety of QZLX treatment.

QZLX alleviated inflammation in DNFB-induced AD-like mice

To evaluate the anti-inflammatory and anti-allergic activities of QZLX, we measured the levels of IgE and AD-related inflammatory cytokines. The QZLX-treated group exhibited significantly lower serum levels of IgE

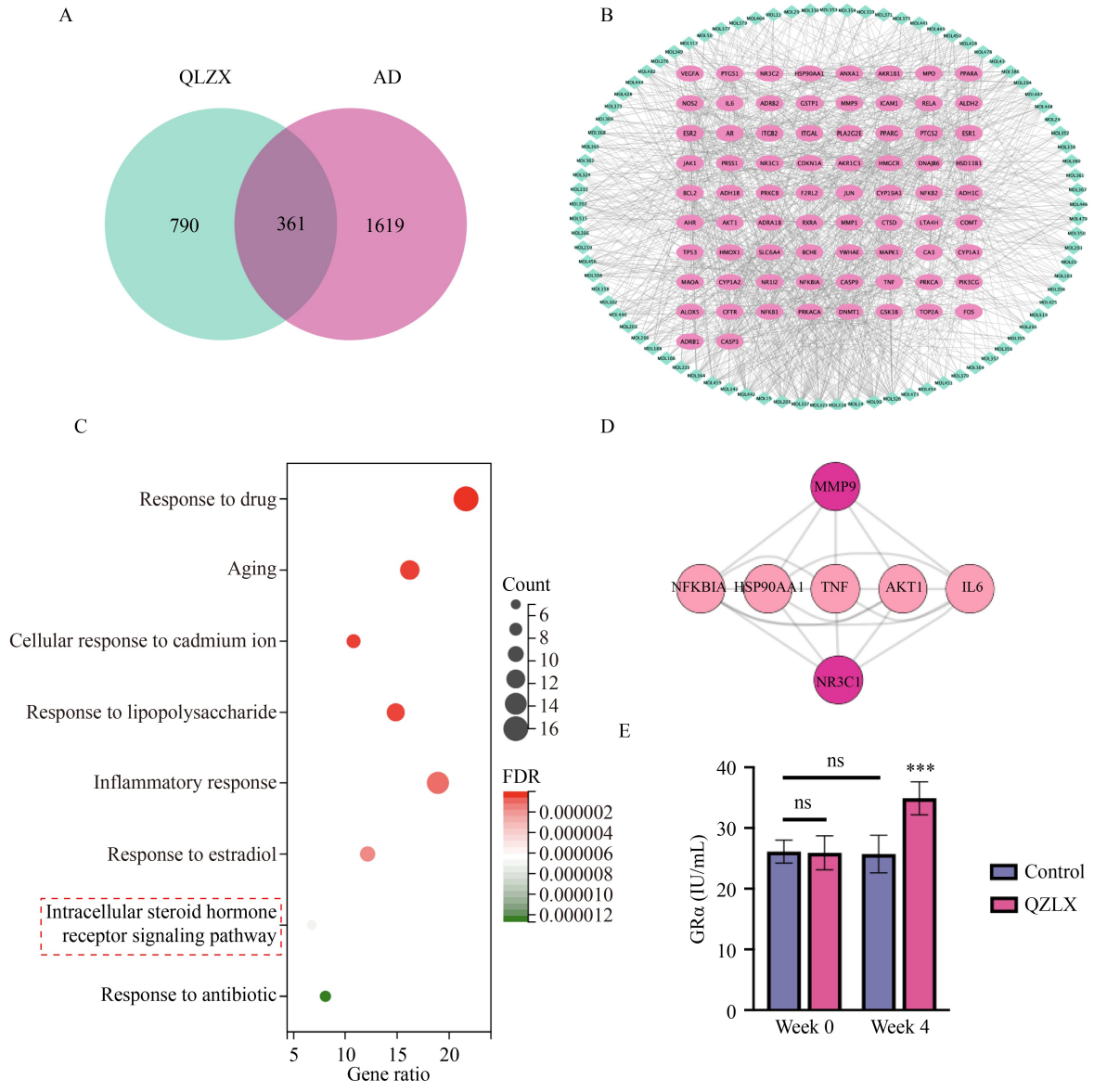

Fig. 3 Network pharmacological analysis and clinical validation. (A) Venn diagram revealing the overlap between AD-related genes and the targets of active ingredients of QZLX. (B) Subnetwork of active compound–target interactions. Green nodes represent the active compounds of QZLX, and pink circles indicate the targets of these compounds. (C) GO enrichment analysis. (D) Core genes identified from the PPI network. (E) Serum expression levels of GRα. Data are presented as mean ± SD, $^{***}P < 0.001$, compared with week 0.

and cytokines, such as IL-4, TNF-α, IL-22, and IL-17, compared with the DNFB group (Fig. 5A–5E). IHC results further demonstrated notable reductions in the NF-κB and CD4 expressions in the skin lesions of QZLX-treated mice compared with those in the DNFB-treated mice (Fig. 5F and 5G). These findings collectively suggest the effectiveness of QZLX in alleviating allergic inflammatory disorders in DNFB-induced AD-like mice.

QZLX increased GRα expression in skin lesions of DNFB-induced AD-like mice

To further validate the effect of QZLX on GRα, we assessed its expression levels in the skin lesions of DNFB-induced AD-like mice. The DNFB group displayed significantly downregulated mRNA and protein levels of GRα compared with the control group. However, QZLX treatment markedly upregulated GRα expression (Fig. 5H and 5I). Based on the results, GRα is a promising therapeutic target for QZLX.

GRα alleviated skin symptoms in DNFB-induced AD-like mice

To elucidate the role of GRα in AD, we administered recombinant GRα protein to DNFB-induced AD-like

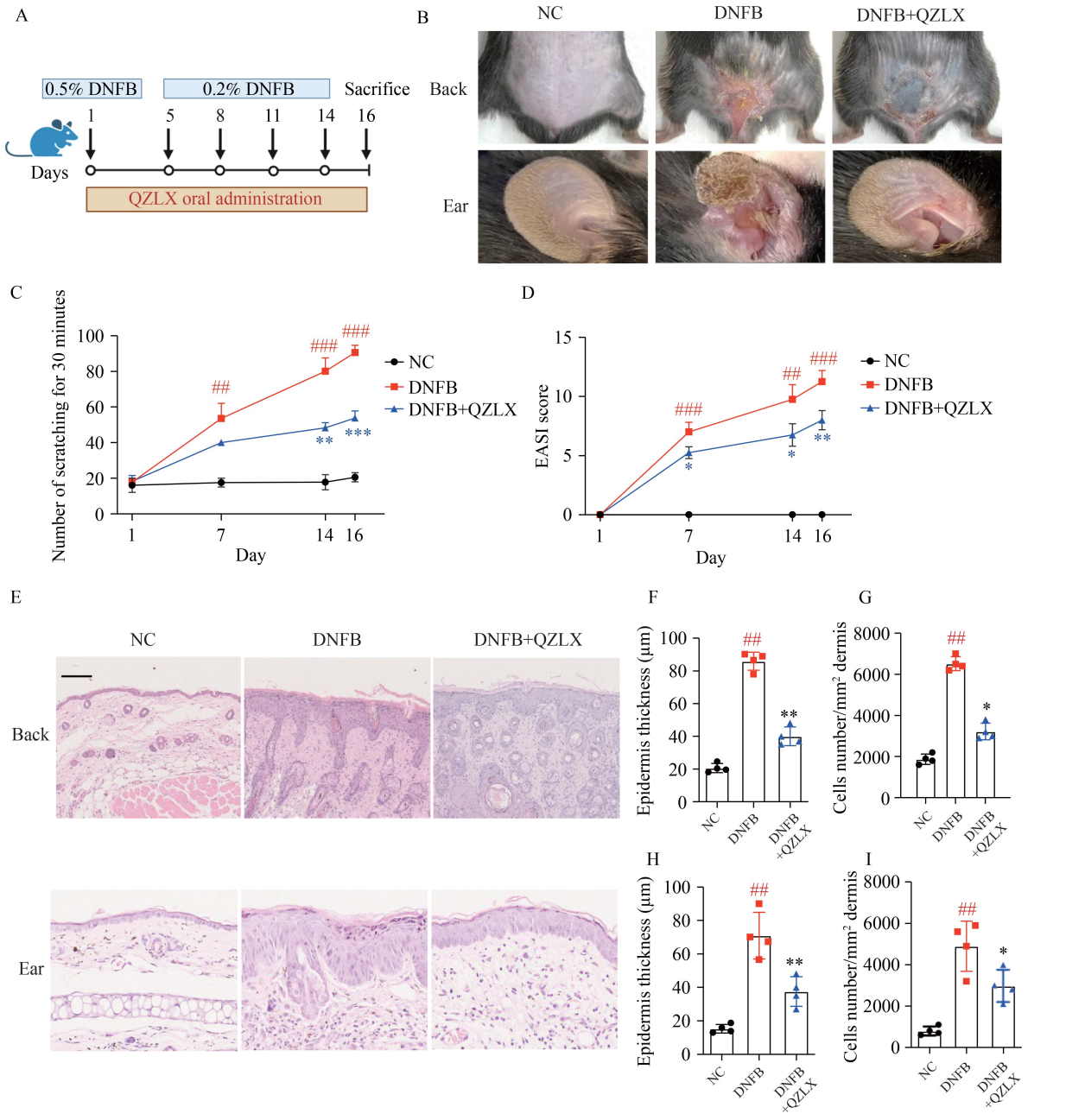

Fig. 4 QZLX attenuated skin symptoms in DNFB-induced AD mice. (A) Schematic of the experimental design for the DNFB-induced AD mouse model. (B) Representative photographs of the back and ear skin. (C) Frequency of scratching behavior. (D) Regional EASI score. (E) Histopathological changes observed in back and ear skin lesions (HE staining, × 200), scale bar: 100 μm. (F) Epidermal thickness of back skin lesions. (G) Quantified dermal inflammatory cells in back skin lesions. (H) Epidermal thickness of ear skin lesions. (I) Quantified dermal inflammatory cells in ear skin lesions. Data are presented as mean ± SD ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, compared with the DNFB group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared with NC group.

mice. The treatment led to a significant alleviation of dermatitis severity and a decrease in scratching behavior (Fig. S4). These results strongly support the hypothesis that upregulation of GRα expression has a beneficial effect on the management of AD symptoms. Despite the improvement of AD symptoms in the GRα treatment group, superior results were observed in the QZLX group.

GRα showed a strong correlation between clinical symptoms and IgE

To evaluate the clinical significance of GRα, we investigated its association with various clinicopathological characteristics. Compared with non-AD individuals, AD patients demonstrated notable histological alterations. These alterations included an augmented epidermal

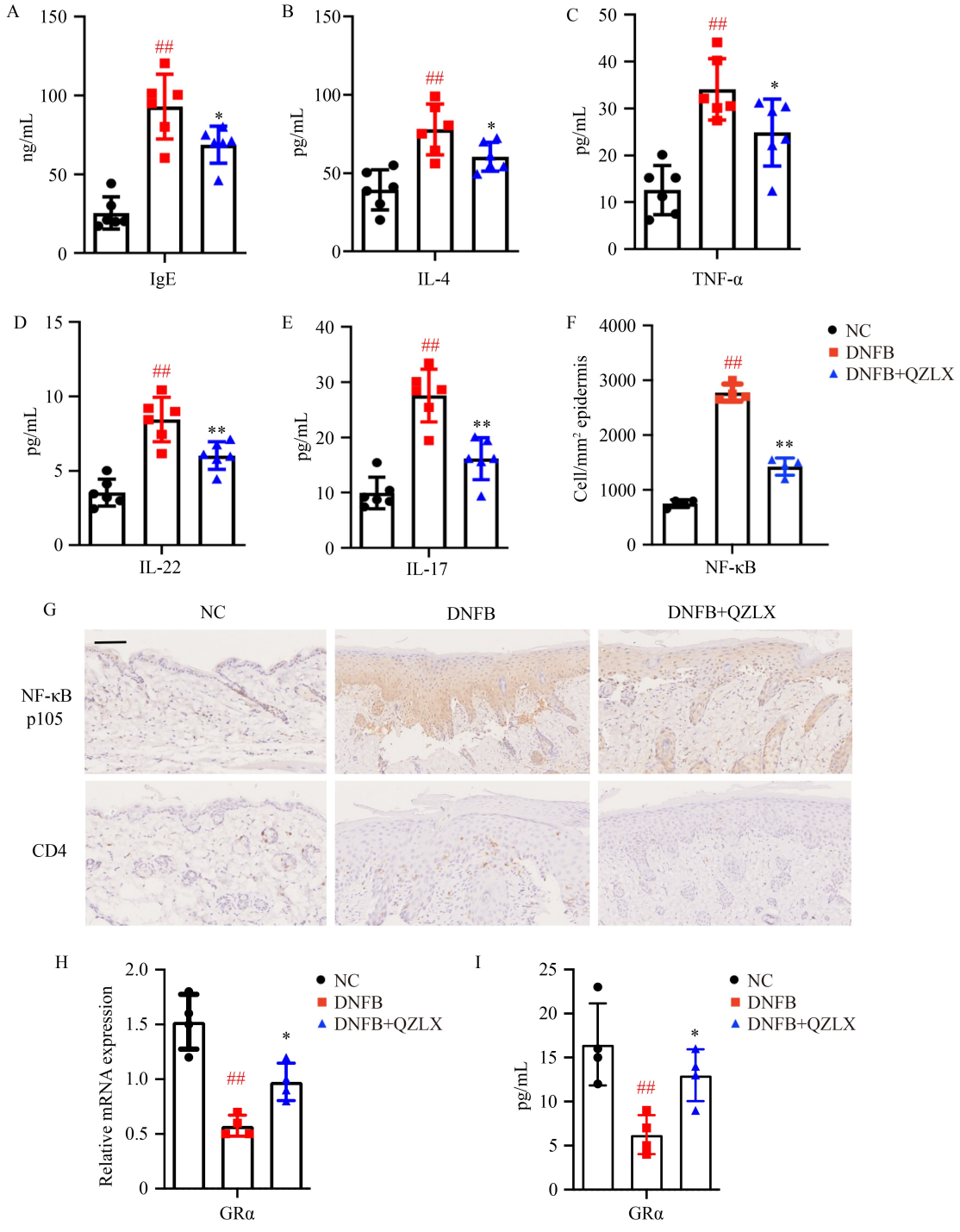

Fig. 5 QZLX alleviated inflammation and increased GRα in DNFB-induced AD mice. (A) IgE, (B) IL-4, (C) TNF-α, (D) IL-22, and (E) IL-17 expression levels in skin lesions. (F) Protein level of NF-κB p105 in epidermal lesions of mice in each group. (G) Representative IHC sections of NF-κB p105 nuclear staining (brown) and CD4 membrane staining (brown) in skin lesions (× 200), scale bar: 100 μm. (H) GRα mRNA level in skin lesions. (I) GRα protein level in skin lesions. Data are presented as mean ± SD (n = 4–6). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the DNFB group, $^{\#\#}P < 0.01$, compared with the NC group.

thickness accompanied with hyperkeratosis, spongiosis, and a heightened infiltration of inflammatory cells in the dermis (Fig. 6A–6C). Immunohistochemically, the prevalence of $CD4^{+}$ membrane-expressing and NF-κB nuclear-positive cells showed marked elevations within the cutaneous lesions of those with AD (Fig. 6A and 6E). In addition, GRα expression within plasmatic nuclei was considerably diminished in AD lesions relative to healthy skin, whereas GRβ-positive cell counts did not exhibit statistical variance (Fig. 6D).

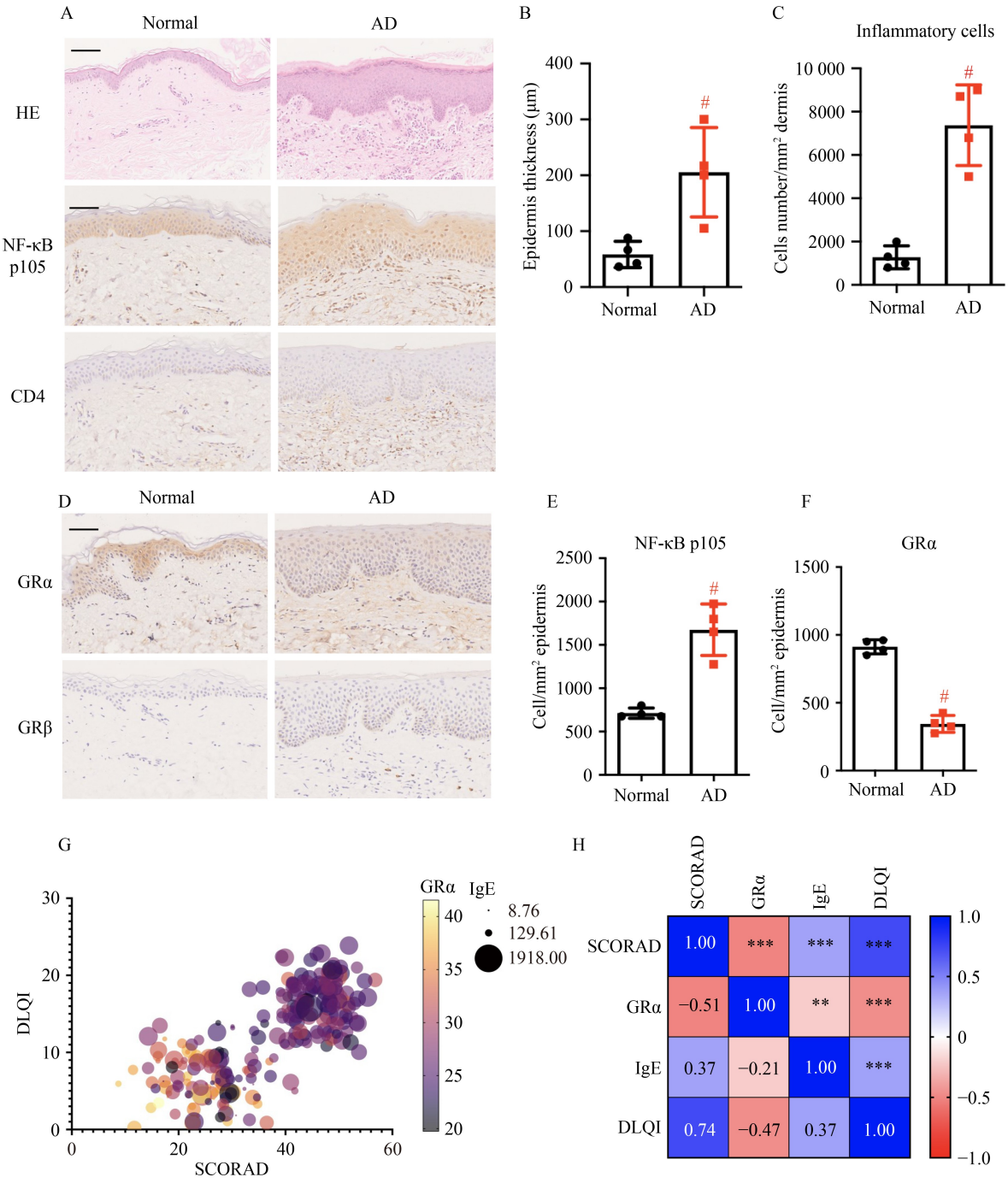

Fig. 6 Clinical correlations of GRα. (A) Representative HE and IHC sections of skin lesions from AD patients and normal volunteers under NF-κB p105 nuclear staining (brown) and CD4 membrane staining (brown) (× 200), scale bar: 100 μm. (B) Epidermal thickness of skin lesions. (C) Number of dermal inflammatory cell. (D) Representative IHC sections of skin lesions with GRα and GRβ nuclear staining (brown) (× 200), scale bar: 100 μm. (E–F) Quantification of NF-κB p105 and GRα cells in skin lesions. Data are presented as mean ± SD (n = 4). $^{*}P < 0.05$, compared with the AD group. (G) Multidimensional bubble plot. Each data point summarized the DLQI, SCORAD, and the serum levels of GRα and IgE for each patient, as indicated by the colors and sizes in the legend (H) Heat map depicting the relationship between GRα and clinical indicators. $^{**}P < 0.01$ and $^{***}P < 0.001$.

In a subsequent examination of the link between serum GRα levels and clinical markers in AD patients, pre- and post-treatment correlations were assessed and visually presented through bubble charts and heatmaps. A discernible inverse correlation was observed between serum GRα levels and AD severity, and it was quantified using the SCORAD, DLQI, and serum IgE levels. GRα expression was reduced prior to therapeutic intervention

but was appreciably augmented posttreatment (Fig. 6G). This trend was corroborated by expression correlation heatmaps, which illustrated potent associations between GRα levels and the aforementioned clinical parameters (Fig. 6H). Collectively, the data indicate that GRα expression may represent a viable biomarker for gauging AD progression and severity.

Discussion

Network pharmacology was integrated with clinical efficacy assessments to elucidate the mechanisms of QZLX and its relation to GRα in the context of AD. The research affirmed QZLX's therapeutic potency and security, and this outcome was corroborated by the findings of clinical trials and animal models. Through these investigative methods, GRα emerged as a central target of QZLX's action. We also evaluated QZLX's effects on inflammatory biomarkers and GRα levels and gauged the *in vivo* effect of its recombinant protein. Crucially, we underscored GRα's clinical prominence by linking it to AD severity. Collectively, the data suggest that QZLX mitigates AD by increasing the GRα expression and curtailing inflammation, and thus, it offers a viable approach for the augmentation of GRα functionality and countering glucocorticoid insensitivity in AD therapy.

Current therapies for AD encompass topical agents, phototherapy, and systemic treatments, such as antihistamines, immunosuppressants, and biologics [28]. Systemic medication becomes necessary when topical treatments and phototherapy prove inadeqate. However, given long-term safety concerns, systemic medication presents an advantage in minimizing the overall dosage of topical glucocorticoids administered. Nonetheless, antihistamines have limited effectiveness in reducing the pruritus associated with AD; immunosuppressants and biologics carry strict contraindications and safety issues for prolonged usage [28]. Therefore, effective and safe AD treatments derived from natural resources must be explored. TCM exhibits evident advantages in this regard and boasts a long history of usage as a supplement and alternative medicine for AD treatment [29]. QZLX, which has been utilized for over three decades, has demonstrated reliable curative effects [14]. To better assess its efficacy and safety in AD patients receiving topical glucocorticoids, we conducted a randomized controlled trial. The trial results reveal that QZLX significantly improved AD symptoms and caused a more pronounced difference in SCORAD and DLQI scores compared with antihistamine medication. However, no statistically significant distinction was observed in the total IgE levels between the two groups. In addition, we detected no impairment of renal or hepatic function and no adverse events following QZLX treatment, which indicates its favorable safety profile in clinical applications. An animal experiment yielded similar results. Moreover, QZLX downregulated the expressions of NF-κB p105 and CD4 in skin lesions of AD-like mice, which suggests its anti-inflammatory activity. AD is currently considered a Th2/Th22-driven inflammatory disease, and Asian and pediatric AD exhibit heightened Th1/Th17 activation alongside a Th2 bias [30]. DNFB-induced AD-like mice displayed a mixed population of Th2, Th17, and Th1 cells [31]. QZLX also inhibited inflammatory cell infiltration in skin lesions and reduced the levels of Th2- (IL-4 and IL-6), Th1- (TNF-α), and Th17-related cytokines (IL-17A). Our findings provide comprehensive evidence supporting the application of QZLX in AD treatment.

AD is a common chronic inflammatory skin disease accompanied with other atopic diseases, such as allergic rhinitis and asthma [32]. Topical glucocorticoids have long been the primary treatment of choice for mild to moderate AD, and systemic glucocorticoids are frequently employed for severe cases [33]. Individuals with comorbid allergic rhinitis and asthma have shown an increased frequency in the use of inhaled or systemic glucocorticoids [34]. Glucocorticoids exert extensive and intricate biological effects, including anti-inflammatory, anti-allergic, and immune suppression properties, which depend on the expression and affinity of GR [35,36]. However, approximately 30% of patients treated with glucocorticoids develop acquired glucocorticoid resistance, which is characterized by a loss of efficacy or desensitization over time [37,38]. Aberrant GR function is a contributing factor to reduced sensitivity to topical glucocorticoids in AD patients [9,12,39]. After oral methylprednisolone therapy, AD patients exhibited significantly fewer GR binding sites in peripheral mononuclear leukocytes, and the GR affinity remained unchanged [40]. Specifically, the expression of GRα, a splicing variant of human GR, was markedly decreased in glucocorticoid-resistant AD patients, whereas that of GRβ was increased. GRα isoforms translocate to the nucleus and modulates the expressions of glucocorticoid-responsive genes by binding to specific glucocorticoid response sites [38]; GRβ isoforms, acting as natural inhibitors of glucocorticoid activity, do not bind glucocorticoids and block the ligand-mediated transactivation of glucocorticoid-responsive genes via GRα [41].

In our study, skin lesions obtained from AD patients showed a significantly lower expression level of GRα protein compared with normal individuals, consistent with our animal data. Meanwhile, the expression level of GRβ protein did not show statistical significance. Furthermore, we observed the cytoplasmic nuclear positivity of GRα, which indicates the presence of glucocorticoid stimulation. A well-known mechanism underlying the anti-inflammatory effects of

glucocorticoids involves the suppression of NF-κB transcription and downstream inflammation through GRα binding [42]. NF-κB is a ubiquitous transcription factor widely involved in inflammatory disorders and comprises homodimers or heterodimers composed of subunits, including p65 (Rel A), c-Rel, Rel B, p52/p100, and p50/p105 [43,44]. *In vitro* and *in vivo* experiments demonstrated that the inhibition of NF-κB signaling pathways decreased the levels of Th2 chemokines in AD, which ameliorates disease progression [45,46]. Our results reveal the higher expressions of CD4 and NF-κB in AD skin lesions from patients and DNFB-induced AD-like mice, which is consistent with those of previous research [47,48]. The amelioration of AD-like symptoms in mice treated with recombinant GRα protein further supports the importance of GRα regulation in the skin inflammation associated with AD. Given the pivotal role of GRα as a regulator in glucocorticoid activity, it holds promise as a potential therapeutic target for the prevention of glucocorticoid resistance in AD.

Network pharmacology is an efficient method for the prediction of complicated TCM processes, and it has been frequently utilized to predict pharmacological mechanisms and guide pharmacological research. Based on the results of GO enrichments analysis, the intracellular steroid hormone receptor signaling pathway and hub target *NR3C1*, which encodes GR, attracted our attention. To validate these findings, we investigated the GR expression in clinical and animal studies. Our results demonstrate that QZLX treatment increased GRα expression levels in the peripheral blood of AD patients and skin lesions of AD-like mice, consistent with our network pharmacological analysis and previous research on herbal components. Baicalin is the main flavonoid component of QZLX, and it is extensively used in the treatment of skin diseases due to its outstanding bioactivities, including anti-inflammatory properties [49]. This molecule upregulates GRα expression and normalizes GR function by promoting GR phosphorylation [50]. Baicalein, another flavonoid extracted from the root of *Scutellaria baicalensis* Georgi, is consumed as a part of this botanical dietary supplement to reduce inflammation [51]. Baicalein exerts its biological activity via the GR and possesses GR agonist properties [52]. A natural fatty acid found in many plants, oleic acid exhibits a high degree in the component-target network. Oleic acid presents GR-dependent anti-inflammatory activity in skin inflammation [53]. Similarly, ursolic acid, a pentacyclic triterpenoid present in several medicinal plants, possesses multiple biological activities, including anti-inflammatory and antioxidant properties [54]. Ursolic acid activates GR, and its structural resemblance to typical glucocorticoids further supports its potential therapeutic relevance [55]. Collectively, QZLX significantly enhanced GRα expression and GR activity through the synergistic effects of multiple compounds. Therefore, this concoction exhibits potential as a complementary therapeutic approach for AD while also functioning as a preventive agent against glucocorticoid resistance in disease management.

Conclusions

This study involved the comprehensive evaluation of the effects and safety of QZLX on AD through clinical and animal studies. Network pharmacology revealed GR as a central target and demonstrated that QZLX effectively mitigated AD symptoms by enhancing GRα expression and reducing inflammation. These findings highlight the therapeutic potential of QZLX in AD treatment. Moreover, our study not only elucidated the mechanism of action underlying a safe and effective AD treatment but also suggests its potential in addressing glucocorticoid resistance. By integrating clinical efficacy and network pharmacology approaches, our research opens up new avenues for gaining insights into the underlying mechanisms of AD.

However, this work suffered from some limitations. First, the specific active ingredients of QZLX responsible for its therapeutic effects in AD treatment have not been fully validated and necessitates further investigation. Second, although GRα showed an increased expression with QZLX treatment, the precise molecular mechanisms underlying the influence of QZLX on GRα remain unclear and warrant further exploration. Future research should aim at uncovering the precise molecular and cellular pathways utilized by the active components of QZLX in targeting GRα, with a particular emphasis on the improvement of its activity and glucocorticoid sensitivity. In addition, long-term safety and efficacy studies are required to comprehensively assess the potential side effects and durability of QZLX treatment. Addressing these limitations will be a focal point in our future research efforts.

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Compliance with ethics guidelines

Keke Huang, Qingkai Liu, Ruoxi Zhang, Hua Nian, Ying Luo, Yue Luo, Xiaoya Fei, Le Kuai, Bin Li, Yimei Tan, Su Li, and Xin Ma declare that they have no conflicts of interest.

The study was approved by the Institutional Review Board of Yueyang Hospital and the study was performed in accordance with the ethical standards as laid down in the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards. Informed consent was obtained from all patients for being included in the study. All institutional and national guidelines for the care and use of laboratory animals were followed.

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