Treatment of allergic rhinitis with acupoint herbal plaster: an oligonucleotide chip analysis

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Abstract

Background: Allergic rhinitis is regarded as an imbalanced Th1/Th2 cell-mediated response. The present study used microarray analysis to compare gene expression levels between allergic rhinitis patients before and after a series of acupoint herbal plaster applications.

Methods: In this experimental pilot study, volunteers experiencing sneezing, runny nose, and congestion for more than 9 months in the year following initial diagnoses were included after diagnostic confirmation by otolaryngologists to exclude patients with sinusitis and nasal polyps. Patients with persistent allergic rhinitis each received four acupoint herbal plaster treatments applied using the moxibustion technique. Clinical outcomes were evaluated using the Rhinitis Quality of Life Questionnaire (RQLQ). Peripheral blood samples were analyzed using an ImmunoCAP Phadiatop test, and patients were classified as phadiatop (Ph)-positive or -negative. Microarray results were analyzed for genes that were differentially expressed between (1) Ph-positive and -negative patients treated with herbal plaster; and (2) before and after herbal plaster treatment in the Ph-positive patient group. Unsupervised and supervised methods were used for gene-expression data analysis.

Results: Nineteen Ph-positive and four Ph-negative participants with persistent allergic rhinitis were included in the study. RQLQ results indicated that the 19 Ph-positive volunteers experienced improvement in six of seven categories following acupoint herbal plaster treatments, whereas the four Ph-negative participants reported improvement in only two categories. Hierarchical clustering and principle component analysis of the gene expression profiles of Ph-positive and -negative participants indicated the groups exhibited distinct physiological responses to acupoint herbal treatment. Evaluation of gene networks using MetaCore identified that the "Immune response_IL-13 signaling via JAK-STAT" and the "Inflammation_Interferon signaling" were down- and up-regulated, respectively, among Ph-positive subjects.

Conclusions: In this preliminary study, we find that the IL-13 immune response via JAK-STAT signaling and interferon inflammation signaling were down- and upregulated, respectively, in the Ph-positive group. Further studies are required to verify these pathways in Ph-positive patients, and to determine the mechanism of such pathway dysregulation.

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Keywords: Allergic rhinitis, Acupoint herbal plaster, Oligonucleotide chip
Background

Many patients with allergic rhinitis have chosen complementary and alternative medicine (CAM), including traditional Chinese medicine (TCM) or acupuncture [1, 2], as they have found CAM to be more attractive and less invasive [1]. The World Health Organization (WHO) published an article examining CAM therapies for allergic rhinitis and asthma [2], which include major contributions from TCM and deserve our continued study to assess therapeutic efficacies and mechanisms. In addition to acupuncture and TCM to treat allergic rhinitis, acupoint herbal plaster applications have recently been used widely in Taiwan [3–5] and mainland China [6, 7] due to the non-invasive and easy to manipulate nature of these treatments. An herbal plaster is applied with a drug applicator using a technique akin to moxibustion, stimulating the skin at specific acupuncture points [3, 4]. Acupoint herbal plaster methods have been recommended for allergic rhinitis beginning in 2009 [8], and practitioners throughout Taiwan and China use similar approaches in the composition of herbal medicine, the herbal medicine application operating process [9] and what acupoints are used [10]. Clinical research regarding the application of acupoint therapy for allergic rhinitis has increased, and evidence-based methods have validated its efficacy and safety [7, 9–11]. However, the majority of these studies are clinical trials; therefore, the efficacy and mechanisms of acupoint herbal plaster treatment need to be validated via mechanistic, molecular methods [2, 9, 12].

We previously studied the effect of herbal plaster treatment for allergic rhinitis [13]. Ours was the first comprehensive clinical outcome assessment of acupoint herbal plaster therapy for allergic rhinitis using the Rhinoconjunctivitis and Rhinitis Quality of Life Questionnaire (RQLQ) [14]. We showed that acupoint herbal plaster for the treatment of allergic rhinitis is safe, effective, and associated with high compliance rates. Here, we aimed to perform a pilot study for acupoint herbal plaster treatment based on our previous microarray experience. Our laboratory has rich microarray experience that combines the Genomic Medicine Research Core Laboratory (GMRCL) [15], clinicians in the Department of Chinese Medicine at Chang Gung Memorial Hospital, and bioinformatics specialists. We performed chip analysis before and after acupuncture treatment in allergic rhinitis patients [16, 17]. We used cDNA microarray and oligonucleotide microarray analyses to investigate the influence of acupuncture on RNA expression profiles using blood samples from patients with allergic rhinitis. We used the RQLQ and statistical analysis to assess clinical outcomes [14]. The results of our microarray analysis were associated with the RQLQ to obtain our final conclusions.

Following exposure to allergens, allergic rhinitis patients exhibit immunoglobulin E (IgE), mast cell, and T helper (Th2) lymphocyte immune responses related to (1) sensitization and memory, (2) the early phase, and (3) the late phase [18, 19]. The early phase can induce sneezing, nasal itching, runny and congested nasal passages, and other symptoms. The late phase contributes to patient fatigue, malaise, irritability, and other symptoms. Allergic rhinitis is regarded as an imbalanced Th1/Th2 cell-mediated response [20, 21]. Th1 cells primarily secrete IL-2, IFNγ, IL-3, and GM-CSF; whereas Th2 cells secrete IL-3, IL-4, IL-5, IL-10, IL-13, and GM-CSF [22]. Dominant Th2 cytokines can enhance allergen-specific IgE, which plays an important role in allergic inflammation [18, 20]. Studies using DNA microarray have indicated an imbalance in the T-helper cell-mediated immune system in patients with allergic rhinitis [23, 24]. Genes encoding chemokines and their receptors were elevated in this analysis; these genes play important roles in the Th2 response [24, 25].

According to our previous study, peripheral blood samples collected from allergic rhinitis patients before and after acupuncture treatment and analyzed by cDNA microarray analysis indicated an improvement in the counterbalance between pro-inflammatory cytokines derived from Th1 cells and anti-inflammatory cytokines derived from Th2 cells [16]. Nasal allergic reactions in patients with allergic rhinitis were inhibited by Th1 cells and were not promoted by Th2 cells following acupuncture treatment [16]. Although strengthening the Th1 response is regarded as a novel therapeutic target for allergic rhinitis, it has not yet been applied in clinical practice [19, 21]. We have published that acupuncture treatment may be another way to restructure Th1 and Th2 responses in patients with allergic rhinitis [16]. ImmunoCAP Phadiatop is a blood test widely used by ENT specialists in Taiwan to detect serum allergen-specific IgE antibodies [26, 27]. Among normal controls and atopic patients, the frequency of Ph-positive patients was 1 of 47 and 49 of 53, respectively [26]. In our previous study [17], Th1 and Th2 cells were suppressed after acupuncture treatment with group differences between Phadiatop (Ph)-positive and Ph-negative patients regarding gene expression characteristics and physiological responses. Studies have shown that the reduction in allergic inflammation and the restored Th1/Th2 (and Treg/Th2) equilibrium following acupuncture are sustained [17].

In this pilot study, we examined changes in gene expression associated with acupoint herbal plaster for allergic rhinitis. Using microarray, we compared gene expression levels in allergic rhinitis patients before and after a series of acupoint herbal plaster applications. This study applies EBM and supports the use of acupoint herbal therapy to treat allergic rhinitis.
Methods

Acupoint herbal plaster treatment

This pilot study was designed using an intervention model with single group assignment. Allergic rhinitis patients were included after their diagnoses were confirmed, and were treated with four applications of herbal plaster. The clinical portion of this study was conducted at the Department of Acupuncture and Moxibustion, Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital from October 2009 to March 2010. Patients (age, 18–45 y) were eligible who met the following criteria: (1) exhibited sneezing, runny nose, and congestion for more than 9 months of the year [18]; (2) did not take medication in the previous month; and (3) provided written consent to enter a Chang Gung Memorial Hospital Institutional Review Board (IRB)-approved human trial. Patient diagnoses were confirmed by the following clinical and biochemical tests, which were performed by otolaryngologists: (1) physical examination; (2) anterior rhinoscopy; (3) ImmunoCAP Phadiatop (InVitroSight, Phadia AB, Uppsala, Sweden), determination of specified serum IgE antibodies to detect inhalant allergens [26, 27]. Patients were included in the trial after their initial diagnoses were confirmed [18, 28]. Patients with sinusitis or nasal polyps, or those who were unwilling or unable to complete the full course of treatment were excluded from the trial. All included patients were diagnosed with allergic rhinitis that was consistent with persistent allergic rhinitis according to ARIA’s new classification system. The ARIA system includes the following rhinitis symptoms and quality of life variables: duration, which includes intermittent or persistent allergic rhinitis; and nasal allergy symptoms, which must occur more than 4 days per week for 4 months per year to qualify as persistent allergic rhinitis [29, 30].

In total, 23 study patients received acupoint herbal plaster applications every 7–10 days over a 4-week period for a total of 4 applications. The herbal plaster consisted of mustard seed, fumurate, asarum, angelica, cinnamon, and ginger at a ratio of 3:3:2:2:0.5:4, respectively. The treatment was prepared by dissolving the ginger in water and adding the powder to form a plaster. Mixtures were formed into cakes of approximately 1.5 × 1.5 × 0.5 cm³ [13] and were held in position using plastic sheets. The following nine acupoints were selected: Dazhui (GV14), Feishu (BL13, both sides), Gaohuang (BL43, both sides), Shenshu (BL23, both sides), and Pishu (BL20, both sides). Each patching time lasted 1–3 h, depending on the patient’s tolerance. When drug cakes were removed, patients typically exhibited local skin redness and experienced slight burning sensations. Subsequent water exposure, including bathing, was avoided for 1–2 h following treatment to prevent skin aggravation. Patient drug tolerance varies, and adhering the cake for too long occasionally led to blisters. Blisters resulting from this treatment were coated with povidone iodine syrup and were protected with sterile gauze bandages.

Outcome evaluation

Clinical symptoms were indexed as follows: (1) assess symptoms before the first acupoint herbal plaster application, (2) determine rhinoconjunctivitis and rhinitis symptoms at the third and fourth acupoint herbal plaster applications. Clinical outcomes were evaluated using the RQLQ, which has been proven to be effective [14, 31] and includes 28 questions in 7 categories. The RQLQ was designed to measure the impact of rhinitis on quality of life. It considers that allergic rhinitis patients often are troubled by nasal symptoms, eye symptoms, sleep problems, emotional problems, social issues, and other symptoms [14, 29].

ImmunoCAP Phadiatop blood test

Prior to treatment at Chang Gung Memorial Hospital, all 23 allergic rhinitis patients were assessed by clinical pathologists using the ImmunoCAP Phadiatop blood test. Patients were evaluated for the presence of IgE antibodies against the following allergens: Dermatophagoides pteronyssinus, cat dander, dog dander, the German cockroach, and Moulds. Detection of IgE antibodies exceeding 0.35 kUA/L indicated a positive result.

RNA extraction and microarray

Patient peripheral blood samples were obtained in 5-ml volumes at the following 6 times (T0–T5) during the study: (1) before (T0) and 24 h after the first (T1) acupoint herbal plaster application; (2) before (T2) and 24 h after the third (T3) acupoint herbal plaster application; and (3) before the fourth (T4) and 24 h after the fourth (T5) acupoint herbal plaster application.

From each 5-ml blood sample, 2.5-ml aliquots were analyzed by the Clinical Pathology Department of Chang Gung Memorial Hospital for the following: complete blood count/differential count (CBC/DC):total white blood count; differential counts for neutrophils, lymphocytes, monocytes, eosinophils, and basophils; red blood cell count; platelet count; hemoglobin, hematocrit, and erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width [RDW]). Total serum IgE levels were tracked before the first acupoint herbal plaster application and 24 h after the fourth acupoint herbal plaster application. The remaining 2.5-ml blood samples were stored at room temperature in PAXgene Blood RNA collection tubes (Qiagen, Valencia, CA, USA), containing an RNA stabilizer. RNA was extracted from blood samples using the PAXgene Blood RNA System (Qiagen), according to the manufacturer’s recommendations, and samples were stored at −80 °C. RNA samples then were isolated using an RNeasy MinElute kit (Qiagen), and RNA quality and quantity were analyzed using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA).
Owing to the IRB's limitation that no more than 5-ml peripheral blood could be collected from each study volunteer, we were unable to obtain sufficient RNA quantities to analyze individual participants. Therefore, we applied pre-amplification pooled mRNA samples to a single microarray chip, a method that has been used frequently in microarray analysis [23]. Although pooling could potentially confound signals by mixing cell populations and individuals, it avoids variation within individuals [32]. Because a microarray using pooled RNA only identifies genes that change dramatically, this approach highlights the most differentially expressed signaling pathways between diseased and control individuals [25]. In our study, equal quantities of mRNA were pooled from individuals with similar clinical diagnosis and IgE levels, thereby increasing RNA homogeneity. Each pooled sample corresponded to the blood RNA from 2 to 3 patients. Samples were analyzed using a GeneChip Human Genome U133 Plus2 array (Affymetrix, Santa Clara, CA, USA) containing approximately 54,675 probes. Samples from the 23 patients were divided into 7 pooled groups for each of the 6 blood collection time points and were applied to 42 chips.

### Statistical analysis
Changes in RQLQ and IgE were compared to the first time point (T0; before first herbal plaster) via a paired Student's t-test and a Mann Whitney U-test, respectively.

### Microarray data analysis
Unsupervised (hierarchical clustering and principal component analysis) and supervised (Student's t-test) methods have traditionally been used to analyze gene-expression data [33]. In this study, data were analyzed by hierarchical clustering using Cluster and TreeView software [34] with the following parameters: (1) standard deviation > 0.4 as the filtering cutoff point (1852 genes with marked changes selected among 35 arrays); (2) mean-centered genes and normalized genes; and (3) cluster analysis conducted using uncentered correlation of arrays. Cluster and TreeView programs were downloaded from http://bonsai.hgc.jp/~mdehoon/software/cluster. The Student's t-test, Mann-Whitney U-test and PCA were performed using MATLAB version 7.4 and Statistics Toolbox version 3.1 (The MathWorks, Boston, MA, USA). A volcano plot was constructed using MATLAB to identify changes in replicate microarray data [35]. Specifically, the negative log of the p value (~log10[p value]) was plotted on the y-axis, and the log2 ratio of the fold change was plotted on the x-axis.

We evaluated genes that were differentially expressed following acupoint herbal plaster applications (T1, T2, T3, T4, T5, are compared with T0). Changes in specific gene expression before and after treatment could suggest potential immune mechanisms associated with acupoint herbal plaster application. RQLQ results were compared with gene expression differences in the final analysis.

### Network visualization and analysis
The MetaCore analytical suite (GeneGo, St. Joseph, MI, USA) was used to compare differences in gene expression networks [36–39]. MetaCore evaluates systems biology and drug development at the computational level, enabling analyses of human protein–protein interactions and mechanisms using the database. This suite contributes to analyses of regulatory networks and signaling pathway gene groups. To perform a network analysis of gene groups, MetaCore can work from an input list of genes and can randomly assign genes to different nodes to assess the probability of an interacting network [37]. In this study, the list of genes represented on the Affymetrix Human U133 Plus2 array was used as a base gene list to calculate p values using MetaCore procedures. MetaCore uses a hypergeometric model to determine significance [38, 39].

### Results
#### Clinical outcomes of acupoint herbal plaster treatment
An otolaryngologist screened 23 study participants with allergic rhinitis, and the GMRCL conducted oligonucleotide chip experiments. Each participant’s diagnosis of perennial allergic rhinitis also was confirmed using anterior nasal endoscopy. Based on the results of an ImmunoCAP Phadiatop blood test of allergen-specific IgE, the 23 volunteers were classified as either Ph-positive (19 participants) or Ph-negative (4 participants) (Table 1). Assessments of clinical symptoms and IgE indices were performed before the first, third, and after the fourth acupoint herbal plaster application. The RQLQ was used to survey the patients, and the results were statistically analyzed for clinical symptoms [14] (Tables 2 and 3).

In the Ph-positive group, the RQLQ results were compared before the first and after the fourth acupoint herbal plaster treatment. We identified significant improvements in six of the seven domains (activity, non-hay fever symptoms, eye symptoms, practical problems, nasal symptoms, and emotional symptoms) examined by the RQLQ (Tables 2 and 3). In the Ph-negative group, only two categories (nasal symptoms, emotional symptoms) appeared to improve following acupoint treatment. These results suggest that acupoint herbal plaster applications evoke distinct physiological responses in these two patient groups. These findings are consistent with our previous studies regarding acupuncture treatment for allergic rhinitis [16, 17].

Total serum IgE values were compared before the first and after the fourth acupoint herbal plaster application.


Table 1 Comparison of baseline characteristics between Ph-positive and Ph-negative patients before treatment

| Variables          | Ph-positive Mean ± SD | Ph-negative Mean ± SD | p Value |
|--------------------|-----------------------|-----------------------|---------|
| Gender             |                       |                       |         |
| Male               | 10 ± 3                | 3 ± 1                 | 0.60^   |
| Female             | 9 ± 1                 | 1 ± 0                 |         |
| Age                | 32.11 ± 5.37          | 35 ± 3.37             | 0.22    |
| Duration of allergic rhinitis |          |                       |         |
| ≥ 10 years         | 14 ± 3                | 3 ± 1                 |         |
| < 10 years         | 5 ± 1                 | 1 ± 0                 | 0.96^   |
| Activity           | 3.12 ± 1.39           | 3.08 ± 1.32           | 0.66    |
| Sleep              | 1.65 ± 1.09           | 1.58 ± 0.92           | 0.64    |
| Non-hay fever symptoms | 2.39 ± 1.14       | 2.25 ± 1.08           | 0.58    |
| Practical problems | 2.84 ± 1.42           | 2.33 ± 1.61           | 0.38    |
| Nasal symptoms     | 2.78 ± 1.17           | 2.94 ± 1.43           | 0.98    |
| Eye symptoms       | 2.37 ± 1.41           | 1.75 ± 1.14           | 0.24    |
| Emotional symptoms | 2.08 ± 1.15           | 1.38 ± 0.92           | 0.15    |
| Overall score      | 2.46 ± 1.02           | 2.19 ± 0.96           | 0.40    |
| IgE (Baseline)     | 302.12 ± 78.75        | 21.25 ± 7.70          |         |
| IgE (Follow-up)    | 333.61 ± 86.01        | 25.10 ± 10.44         |         |

SD Standard Deviation
Note: *p < 0.05, **p < 0.01 (Mann–Whitney U test)
^Fisher’s exact test

(Table 1). Following the course of herbal plaster treatments, total IgE levels were unchanged in both the Ph-positive and -negative groups (Tables 4 and 5). This is consistent with previous short-term studies by our laboratory [16, 17] and others [40], which found that total serum IgE levels in allergic rhinitis patients treated with TCM did not change.

Table 2 Changes in RQLQ results following the third and fourth herbal plaster (hp) treatments in Ph-positive patients

| Area of RQLQ | Baseline score | After 3rd hp | P value (3rd hp vs. baseline) | After 4th hp | P value (4th hp vs. baseline) |
|--------------|----------------|-------------|------------------------------|-------------|------------------------------|
| Activity     | 3.12 ± 2.56    | 0.1322      | 2.09                         | 0.0002**    |
| Sleep        | 1.65 ± 1.58    | 0.8488      | 1.35                         | 0.0804      |
| Non-hay fever symptoms | 2.39 ± 2.02 | 0.1465     | 1.64                         | 0.0012**    |
| Practical problems | 2.84 ± 2.39 | 0.1549     | 2.05                         | 0.0018**    |
| Nasal symptoms | 2.78 ± 2.30    | 0.1006      | 1.92                         | 0.0000**    |
| Eye symptoms  | 2.37 ± 1.57    | 0.0330*     | 1.29                         | 0.0066**    |
| Emotional symptoms | 2.08 ± 1.62     | 0.0634     | 1.33                         | 0.0010**    |
| Overall score | 2.46 ± 2.00    | 0.0635      | 1.67                         | 0.0000**    |

Paired Student’s t-test; n = 19, *p < 0.05 **p < 0.01

Ph-positive and Ph-negative allergic rhinitis patients exhibit distinct gene expression profiles following acupoint herbal plaster treatment

Since Ph-positive and Ph-negative groups exhibited different clinical outcomes, we explored the gene expression profiles of these two patient groups following acupoint herbal plaster treatment. Total RNA was extracted from peripheral blood samples at each of the 6 time points analyzed (23 patients, 138 RNA samples total). Because of insufficient blood RNA quantities (1–2 μg/subject), we pooled sets of 2–3 RNA samples from subjects with similar clinical indices, resulting in seven pooled RNA samples for each of the six time points. The 42 pooled RNA samples were applied to GeneChip Human Genome U133 Plus 2.0 arrays. Patient and sample information are detailed in Table 6.

To estimate the effects of acupoint herbal plaster treatment, the gene expression level at each treatment point was subtracted from the first time point (T0; before herbal plaster treatment). After filtering the low-intensity non-significant genes (standard deviation < 0.4), 1852 genes remained for analysis with non-supervised hierarchical clustering methods. We identified distinct gene expression profiles in Ph-positive and -negative patients using a hierarchical approach (Fig. 1a). We further analyzed the correlation matrix for all 35 samples using a PCA [41]. The three-dimensional plot of the first three principal components by the matrix containing 80 % of the information

Table 3 Changes in RQLQ results following the third and fourth herbal plaster (hp) treatments in Ph-negative patients

| Area of RQLQ | Baseline score | After 3rd hp | P value (3rd hp vs. baseline) | After 4th hp | P value (4th hp vs. baseline) |
|--------------|----------------|-------------|------------------------------|-------------|------------------------------|
| Activity     | 3.08 ± 1.58    | 0.0577      | 1.33                         | 0.0800      |
| Sleep        | 1.58 ± 1.58    | 1.0000      | 1.17                         | 0.3677      |
| Non-hay fever symptoms | 2.25 ± 1.79     | 0.3477    | 1.21                         | 0.0564      |
| Practical problems | 2.33 ± 1.67     | 0.3994    | 1.33                         | 0.1135      |
| Nasal symptoms | 2.94 ± 2.06    | 0.1881      | 1.31                         | 0.0065**    |
| Eye symptoms  | 1.75 ± 1.19    | 0.4338      | 1.19                         | 0.4594      |
| Emotional symptoms | 1.38 ± 1.06     | 0.5551    | 0.69                         | 0.0486*     |
| Overall score | 2.19 ± 1.56    | 0.1940      | 1.18                         | 0.0371*     |

Paired Student’s t-test; n = 4, *p < 0.05 **p < 0.01

Table 4 Changes in total IgE levels following the fourth herbal plaster (hp) treatment in Ph-positive patients

| No. | Baseline Mean ± SD | Follow-up Mean ± SD | P value^ |
|-----|-------------------|---------------------|----------|
| IgE | 19                | 302.12 ± 78.75      | 333.61 ± 86.01 | 0.085    |

SD Standard Deviation
^Mann–Whitney U test
is shown in Fig. 1b. This analysis indicated that the Ph-positive and -negative groups were distinct in their responses to acupoint herbal plaster treatment. Because the hierarchical clustering and PCA suggested that the M4-2 and M4-4 samples were outliers in the Ph-positive group, these samples were excluded from further analysis.

Since the clinical outcomes (RQLQ) after treatment in the Ph-positive and -negative groups differed, we explored the gene expression profiles for these two groups in response to acupoint herbal plaster application. We used a volcano plot to obtain an overview of the 1852 filtered genes (Fig. 2a), and we selected 89 genes that exhibited fold-changes exceeding $2^{0.75} = 1.682$ and were differentially expressed following acupoint herbal plaster treatment. We used a Student's $t$-test to compare these genes with Ph-negative group. We selected 47 genes that exhibited fold changes (vs. T0) of $2^{0.4} = 1.320$ (Fig. 3 and Table 9). Globally, most genes were down-regulated (45/47) after herbal plaster treatment. This result was consistent with our previous report that most genes were down-regulated after acupuncture treatment in Ph-positive allergic rhinitis patients [17].

These 45 genes then were input to the MetaCore reaction pathways analysis. The data indicated that Ph-positive allergic rhinitis patients who received acupoint herbal plaster applications significantly induced several pathways ($p < 0.01$; Table 10). Among the 45 down-regulated genes, pathway analysis identified significant involvement of the “Oxidative phosphorylation pathway” ($p < 0.0001$). Network analysis also identified “Protein folding Response to unfolded proteins,” “Immune response Antigen presentation,” and “Immune response Phagosome in antigen presentation” as significant ($p < 0.001$) relative to the 45 down-regulated genes.

### Discussion

Allergic rhinitis likely results from an imbalance in the Th1 and Th2 cell-mediated inflammatory responses [20, 21]. In addition to the hygiene hypothesis causing deviation of the Th1 and Th2 balance and reduced immune suppression, investigators have implicated decreases in T-regulatory (Treg) activity in allergy diseases [42, 43]. People suffering from allergies, usually have a reduced Th1 reaction and a predominant Th2 response. Th1 cells tend to decrease in patients with allergic rhinitis, whereas Th2 cells were significantly increased. Significant deviations from the normal Th1/Th2 ratio may be associated with the incidence of allergic diseases [18, 20, 44]. A study examining allergic inflammation that focused on Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) reported that these cytokines recruited cells that induced allergic inflammation via chemokine secretion [44]. Few reports have described human allergic inflammation with respect to cytokine antagonists [19, 21, 45].

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### Table 5 Changes in patient total IgE levels following the fourth herbal plaster (hp) treatment in Ph-negative patients

| No. | Baseline Mean ± SD | Follow-up Mean ± SD | $P$ value$^*$ |
|-----|-------------------|---------------------|--------------|
| IgE | 21.25 ± 7.70      | 25.10 ± 10.44       | 0.63         |

SD Standard Deviation

$^*$Mann–Whitney U-test

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### Table 6 Pooling strategy for RNA samples. The first number in each cell indicates the group type, and the second indicates the time point (T0–T5 correspond to 1–6, respectively). A total of 42 chips were used. M, microarray chip

| Group Type | Before 1st herbal plaster (hp) (T0) | After 1st hp 24 h (T1) | Before 3rd hp 24 h (T3) | After 3rd hp 24 h (T3) | After 4th hp 24 h (T4) | After 4th hp 24 h (T5) |
|------------|-----------------------------------|------------------------|-------------------------|------------------------|------------------------|------------------------|
| Ph(+)      | M1-1                              | M1-2                   | M1-3                    | M1-4                   | M1-5                   | M1-6                   |
| Ph(+)      | M2-1                              | M2-2                   | M2-3                    | M2-4                   | M2-5                   | M2-6                   |
| Ph(+)      | M3-1                              | M3-2                   | M3-3                    | M3-4                   | M3-5                   | M3-6                   |
| Ph(+)      | M4-1                              | M4-2                   | M4-3                    | M4-4                   | M4-5                   | M4-6                   |
| Ph(+)      | M5-1                              | M5-2                   | M5-3                    | M5-4                   | M5-5                   | M5-6                   |
| Ph(+)      | M6-1                              | M6-2                   | M6-3                    | M6-4                   | M6-5                   | M6-6                   |
| Ph(−)      | M7-1                              | M7-2                   | M7-3                    | M7-4                   | M7-5                   | M7-6                   |
Although strengthening the Th1 response is regarded as a novel therapeutic approach for allergic rhinitis, this method has not been applied clinically [19, 21]. A restructuring of the Th1 and Th2 responses in patients with allergic rhinitis may be accomplished with acupuncture [16, 17]. Studies have shown that acupuncture treatment of allergic inflammation can maintain the equilibrium between Th1 and Th2 cells and between Tregs and Th2 cells [16, 17]. Many patients choose acupoint herbal plaster treatments for allergic rhinitis in Taiwan [3–5] and mainland China [6, 7]. We previously examined the efficacy of acupuncture treatment of allergic inflammation by determining the expression levels of specific genes [13]. The present study is the first to apply the RQLQ to comprehensively assess the effects of acupoint herbal plaster on allergic rhinitis symptoms. Our results suggest that acupoint herbal plaster is a safe, effective, and convenient treatment for allergic rhinitis. A comparison of baseline characteristics before treatment between Ph-positive and Ph-negative patients showed no differences, with the exception of total IgE levels (Table 1). The RQLQ results after the fourth treatment of 19 Ph-positive patients indicated symptom improvements in six of seven categories (activity, non-hay fever symptoms, practical problems, nasal symptoms, eye symptoms, emotional symptoms; Tables 2 and 3). In contrast, the four Ph-negative volunteers (−) reported symptom improvements in only two categories (nasal symptoms, emotional symptoms; Tables 2 and 3). These results are similar to those found in our previous report on acupuncture treatment for allergic rhinitis [16, 17]; however,
Table 7 The 89 genes that were differentially expressed between Ph-positive and Ph-negative patients with allergic rhinitis following treatment with acupoint herbal paste

| ID          | Gene Symbol | Gene Title                                         | Fold change | P value   |
|-------------|-------------|----------------------------------------------------|-------------|-----------|
| 1552288_at  | CILP2       | cartilage intermediate layer protein 2            | 1.45         | 3.4E-05   |
| 1556590_s_at| NA          | NA                                                 | 1.32         | 1.4E-04   |
| 1557195_at  | NA          | NA                                                 | 1.31         | 8.8E-04   |
| 1557761_s_at| LOC400794   | hypothetical LOC400794                             | 1.31         | 3.9E-06   |
| 1562216_at  | NA          | NA                                                 | 1.30         | 1.7E-04   |
| 1565913_at  | NA          | NA                                                 | 1.21         | 4.2E-10   |
| 1566134_at  | CARHSP1     | Calcium regulated heat stable protein 1, 24 kDa    | 1.20         | 6.5E-04   |
| 1566964_at  | NA          | NA                                                 | 1.18         | 3.4E-04   |
| 1567240_x_at| OR2L2       | olfactory receptor, family 2, subfamily L, member 2| 1.11         | 7.1E-03   |
| 1569482_at  | NA          | NA                                                 | 1.08         | 8.8E-03   |
| 200038_s_at | RPL17       | ribosomal protein L17                              | 1.08         | 7.4E-04   |
| 200082_s_at | RPS7        | ribosomal protein S7                               | 1.06         | 3.7E-03   |
| 200705_s_at | EEF1B2      | eukaryotic translation elongation factor 1 beta 2  | 1.02         | 2.6E-03   |
| 200986_at   | SERPING1    | serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 | 1.02 | 2.0E-03 |
| 201699_at   | PSMC6       | proteasome (prosome, macropain) 26S subunit, ATPase, 6 | 1.01 | 4.0E-03 |
| 202086_at   | MX1         | myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (murine) | 1.01 | 7.2E-09 |
| 202411_at   | IFI27       | interferon, alpha-inducible protein 27             | 1.00         | 7.8E-05   |
| 202635_s_at | POLR2K      | polymerase (RNA II (DNA directed) polypeptide K, 7.0 kDa) | 0.99 | 1.4E-03 |
| 204286_s_at | PMAIP1      | phorbol-12-myristate-13-acetate-induced protein 1   | 0.98         | 5.5E-09   |
| 204415_at   | IFI6        | interferon, alpha-inducible protein 6              | 0.97         | 7.6E-05   |
| 204439_at   | IFI44L      | interferon-induced protein 44-like                 | 0.96         | 2.0E-03   |
| 204732_s_at | TRIM23      | tripartite motif-containing 23                     | 0.93         | 1.7E-03   |
| 205849_s_at | UQCRB       | ubiquinol-cytochrome c reductase binding protein   | 0.91         | 7.7E-03   |
| 205914_s_at | GRIN1       | glutamate receptor, ionotropic, N-methyl D-aspartate 1 | 0.90 | 1.5E-03 |
| 206584_at   | LY96        | lymphocyte antigen 96                             | 0.90         | 1.2E-04   |
| 207723_s_at | KLRC3       | killer cell lectin-like receptor subfamily C, member 3 | 0.88 | 3.6E-04 |
| 208792_s_at | CLU         | clusterin                                          | 0.88         | 3.5E-03   |
| 209160_at   | AKR1C3      | aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, t hydroxylation) | 0.88 | 3.9E-06 |
| 209651_at   | TGFBI1      | transforming growth factor beta 1 induced transcript | 0.86 | 1.0E-03 |
| 209732_at   | CLEC2B      | C-type lectin domain family 2, member B            | 0.86         | 3.7E-03   |
| 209743_s_at | ITCH        | itchy E3 ubiquitin protein ligase homolog (mouse)  | 0.85         | 2.3E-03   |
| 209795_at   | CD69        | CD69 molecule                                      | 0.85         | 3.9E-08   |
| 210103_s_at | FOXA2       | forkhead box A2                                   | 0.84         | 9.9E-04   |
| 210432_s_at | SCN3A       | sodium channel, voltage-gated, type III, alpha subunit | 0.83 | 2.8E-04 |
Table 7 The 89 genes that were differentially expressed between Ph-positive and Ph-negative patients with allergic rhinitis following treatment with acupoint herbal paste (Continued)

| Gene ID      | Gene Symbol | Description                                                                 | Log2FoldChange | P-value    |
|--------------|-------------|------------------------------------------------------------------------------|----------------|------------|
| 210548_at    | CCL23       | chemokine (C-C motif) ligand 23                                              | 0.83           | 8.5E-05    |
| 210639_s_at  | ATG5        | ATG5 autophagy related 5 homolog (S. cerevisiae)                            | 0.82           | 1.7E-07    |
| 210873_x_at  | APOBEC3A    | apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A         | 0.82           | 2.7E-04    |
| 211968_s_at  | HSP90AA1    | heat shock protein 90 kDa alpha (cytosolic), class A member 1               | 0.81           | 8.0E-05    |
| 212270_x_at  | RPL17       | ribosomal protein L17                                                       | 0.81           | 2.9E-04    |
| 212537_x_at  | RPL17       | ribosomal protein L17                                                       | 0.78           | 1.6E-03    |
| 213226_at    | CCNA2       | cyclin A2                                                                   | 0.78           | 4.2E-03    |
| 214070_s_at  | ATP10B      | ATPase, class V, type 10B                                                    | 0.78           | 3.3E-03    |
| 215101_s_at  | CXCL5       | chemokine (C-X-C motif) ligand 5                                             | 0.78           | 6.0E-03    |
| 215394_at    | PIK3C3      | phosphoinositide-3-kinase, class 3                                           | 0.77           | 6.3E-10    |
| 215646_s_at  | VCAN        | versican                                                                    | 0.77           | 4.3E-04    |
| 216412_x_at  | LOC100290557| similar to hCG91935                                                         | 0.77           | 1.3E-03    |
| 216834_at    | RG51        | regulator of G-protein signaling 1                                          | 0.76           | 2.3E-03    |
| 217915_s_at  | RSL24D1     | ribosomal L24 domain containing 1                                            | 0.76           | 9.8E-04    |
| 219519_s_at  | SIGLEC1     | sialic acid binding Ig-like lectin 1, sialoadhesin                          | 0.76           | 4.7E-04    |
| 219551_at    | EAF2        | ELL associated factor 2                                                      | 0.76           | 3.1E-03    |
| 220141_at    | C11orf63    | chromosome 11 open reading frame 63                                         | 0.75           | 5.8E-03    |
| 220184_at    | NANOG       | Nanog homeobox                                                              | −0.75          | 3.9E-03    |
| 220646_s_at  | KLRF1       | killer cell lectin-like receptor subfamily F, member 1                       | −0.75          | 3.9E-11    |
| 220827_at    | NA          | NA                                                                          | −0.76          | 1.6E-03    |
| 222229_s_at  | RPL26       | ribosomal protein L26                                                        | −0.77          | 5.9E-05    |
| 222465_at    | RSL24D1     | ribosomal L24 domain containing 1                                            | −0.78          | 1.5E-03    |
| 223963_s_at  | IGF2BP2     | insulin-like growth factor 2 mRNA binding protein 2                          | −0.79          | 2.5E-04    |
| 224293_at    | TTTY10      | testis-specific transcript, Y-linked 10 (non-protein coding)                | −0.79          | 8.6E-03    |
| 225541_at    | RPL22L1     | ribosomal protein L22-like 1                                                 | −0.80          | 4.7E-03    |
| 226344_at    | ZMAT1       | zinc finger, matrin type 1                                                   | −0.81          | 1.4E-04    |
| 227454_at    | TAO1        | TAO kinase 1                                                                 | −0.81          | 9.4E-04    |
| 227766_at    | LIG4        | ligase IV, DNA, ATP-dependent                                                | −0.82          | 9.9E-03    |
| 228174_at    | SCAI        | suppressor of cancer cell invasion                                          | −0.83          | 8.5E-03    |
| 228439_at    | BATF2       | basic leucine zipper transcription factor, ATF-like 2                        | −0.83          | 9.6E-03    |
| 228970_at    | ZBTB8OS     | zinc finger and BTB domain containing 8 opposite strand                    | −0.86          | 1.1E-07    |
| 229431_at    | RFXAP       | regulatory factor X-associated protein                                       | −0.86          | 8.8E-03    |
| 229437_at    | MIR155HG    | MIR155 host gene (non-protein coding)                                       | −0.87          | 4.5E-04    |
| 229893_at    | FRMD3       | FERM domain containing 3                                                    | −0.89          | 7.0E-03    |
| 229910_at    | SHE         | Src homology 2 domain containing E                                           | −0.89          | 2.0E-03    |
| 230153_at    | NEK9        | NIMA (never in mitosis gene a)-related kinase 9                             | −0.89          | 6.2E-09    |
| 231014_at    | TRIM50      | tripartite motif-containing 50                                               | −0.89          | 4.8E-03    |
| 231038_s_at  | NA          | NA                                                                          | −0.92          | 8.1E-03    |
the herbal plaster treatment was noninvasive and easy to apply. The degree of symptom improvement among Ph-positive allergic rhinitis patients was different with the Ph-negative group, indicating that the acupoint herbal plaster treatment in these patient groups evoked distinct physiological responses. Due to its preliminary nature, this study has some limitations including the lack of a control group or a safety assessment.

In this study, the average total serum IgE levels tended to increase in Ph-positive and -negative groups following the fourth herbal plaster treatment, but the changes were not statistically significant (Table 4 and 5). This study has some limitations including the lack of a control group or a safety assessment.

Table 7 The 89 genes that were differentially expressed between Ph-positive and Ph-negative patients with allergic rhinitis following treatment with acupoint herbal paste (Continued)

| Gene ID       | Symbol          | Description                                      | Log2 fold change | p-value |
|---------------|-----------------|--------------------------------------------------|------------------|---------|
| 231484_at     | NA              | NA                                               | −0.92            | 1.5E-04 |
| 231688_at     | MMP8            | matrix metalloproteinase 8 (neutrophil collagenase) | −0.93            | 6.4E-03 |
| 231975_s_at   | MIER3           | mesoderm induction early response 1, family member 3 | −0.94            | 1.9E-03 |
| 233015_at     | MBNL1           | muscleblind-like (Drosophila)                    | −0.96            | 3.7E-03 |
| 235762_at     | TAS2R14         | taste receptor, type 2, member 14                | −0.97            | 8.7E-05 |
| 236495_at     | NA              | NA                                               | −0.97            | 8.1E-10 |
| 236666_s_at   | LRRRC10B        | leucine rich repeat containing 10B               | −0.98            | 1.1E-05 |
| 237689_at     | SARS            | Seryl-tRNA synthetase                            | −1.00            | 1.8E-03 |
| 238174_at     | NA              | NA                                               | −1.01            | 6.3E-03 |
| 238918_at     | NA              | NA                                               | −1.06            | 1.7E-03 |
| 239655_at     | NA              | NA                                               | −1.07            | 4.2E-03 |
| 239819_at     | NA              | NA                                               | −1.08            | 1.4E-04 |
| 240145_at     | NA              | NA                                               | −1.10            | 5.3E-03 |
| 240262_at     | NA              | NA                                               | −1.11            | 4.2E-05 |
| 240652_at     | NA              | NA                                               | −1.12            | 8.0E-10 |
| 240866_at     | NA              | NA                                               | −1.26            | 3.8E-03 |
| 242625_at     | RSAD2           | radical S-adenosyl methionine domain containing 2 | −1.43            | 2.0E-03 |

NA Not Available

*Fold change (Log2 ratio)

Table 8 Metacore process map for the 89 genes that were differentially expressed between Ph-positive and Ph-negative patients with allergic rhinitis following acupoint herbal paste treatment

| Maps                               | P-value | Filter Genes a | Map genes b |
|------------------------------------|---------|----------------|-------------|
| DNA damage_NHEJ mechanisms of DSBs repair | 1.4E-02 | 1 (LIG4)       | 19          |
| Neurophysiological process_Bitter taste signaling | 2.0E-02 | 1 (TAS2R14)    | 28          |
| Apoptosis and survival_Granzyme A signaling | 2.1E-02 | 1 (LIG4)       | 30          |
| Cell cycle_Role of Nek in cell cycle regulation | 2.3E-02 | 1 (NEK9)       | 32          |
| Development_Role of Activin A in cell differentiation and proliferation | 2.9E-02 | 1 (NANOG)      | 40          |
| Immune response_IL-13 signaling via JAK-STAT | 3.1E-02 | 1 (MMP8)       | 44          |

| Maps                               | P-value | Filter Genes a | Map genes b |
|------------------------------------|---------|----------------|-------------|
| Inflammation_Interferon signaling | 1.1E-02 | 3 (IFN,IFN2, MX1) | 110         |
| Autophagy_Autophagy                | 2.3E-02 | 2 (PIK3C3,ATG5) | 55          |
| Cell cycle_S phase                 | 2.6E-02 | 2 (HSP90AA1, CCNA2) | 149         |

aNumber of filter genes in the map
bNumber of genes in the map
The result is similar to that of our previous acupuncture study [16, 17] and may indicate that reducing total IgE synthesis is not the primary mechanism of acupoint herbal plaster treatment of allergic rhinitis.

The Ph-positive and -negative groups exhibited different gene expression trends after acupoint herbal plaster treatment (Fig. 2 and Table 7). This supports the results of the RQLQ, and indicates that the patient groups respond differently to acupoint herbal plaster.

Pathway analysis of the differentially expressed genes indicated that “Immune response_IL-13 signaling via JAK-STAT” and “Inflammation_Interferon signaling” pathways corresponded to down- and up-regulated genes, respectively, between Ph-positive and Ph-negative patients (Fig. 2b and Table 8). Since a Th1/Th2 cytokine imbalance contributes to the etiology and pathogenesis of allergic rhinitis, understanding the mechanisms of this disease will help to find novel targets for therapy. Th1 cells secrete primarily IL-2, IFNγ, IL-3, and GM-CSF, whereas Th2 cells secrete IL-3, IL-4, IL-5, IL-10, IL-13, and GM-CSF [22]. Cytokines released after activation of T-cell receptors interact with cytokine receptors on mononuclear cells and activate these cells via the JAK-STAT (Janus kinase and signal transducers and activators of transcription) pathway. The JAK-STAT pathway is involved in histamine-mediated regulation of the Th2 cytokines IL-5, IL-10, and IL-13, and of the Th1 cytokine IFNγ [22]. IL-13 plays a central role in the promotion of an allergic inflammatory eosinophilic reaction in allergic diseases via IgE isotype switching. IFNγ down-regulates the secretion of certain Th2 cytokines [22]. Local administration of IFNγ in mice prevented antigen-induced eosinophil infiltration into the trachea and normalized airway function. However, recombinant subcutaneous administration of IFNγ had no benefit in the treatment of steroid-dependent asthma [22]. Pathways that down-regulated IL-13 signaling via JAK-STAT and upregulated Interferon signaling pathways were differentially expressed between Ph-positive and Ph-negative patients with allergic rhinitis after acupoint herbal paste treatment; however, further studies are necessary to confirm these results.

Several pathways were significantly induced in Ph-positive allergic rhinitis patients who received acupoint herbal plaster applications. Phagosomal immune response in antigen presentation was noted due to an immune response to the herbal plaster treatment (Table 10). Macrophages function to clear infectious particles, and this process involves engulfing microbes into phagosomes where they are lysed and degraded. Phagosomes are pivotal in linking both the innate and adaptive immune responses [46]. Phagosomal proteins regulated by IFNγ include proteins expected to alter phagosome maturation, enhance microbe degradation, trigger the macrophage immune response, and promote antigen loading on major histocompatibility complex (MHC) class I molecules [46]. IFNγ delays phagosomal acquisition of lysosomal hydrolases and peptidases to aid in antigen presentation, which is dependent on phagosomal networks of the actin cytoskeleton and vesicle-trafficking proteins, as well as Src kinases and calpain proteases [47].

In this preliminary study, Ph-positive patients with allergic rhinitis who received acupoint herbal plaster treatments manifested gene expression changes involved in the “Immune response_IL-13 signaling via JAK-STAT” pathway. These patients reported improved clinical
Table 9 The 47 genes that were differentially expressed as compared to the first time point (T0; before herbal plaster treatment in the Ph-positive group)

| ID     | Gene Symbol | Gene Title                                                                 | Fold change | P value   |
|--------|-------------|-----------------------------------------------------------------------------|-------------|-----------|
| 211969_at | HSP90AA1     | heat shock protein 90 kDa alpha (cytosolic), class A member 1               | −0.62       | 5.4E-10   |
| 224567_x_at | MALAT1      | Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding) | −0.62       | 3.3E-06   |
| 226675_s_at | MALAT1      | Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding) | −0.58       | 1.0E-04   |
| 216563_at | ANKRD12     | Ankyrin repeat domain 12                                                    | −0.58       | 2.8E-04   |
| 222465_at | RSL24D1     | ribosomal L24 domain containing 1                                            | −0.58       | 1.6E-06   |
| 204732_s_at | TRIM23      | tripartite motif-containing 23                                               | −0.56       | 2.8E-07   |
| 201304_at | NDUFA5      | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, S, 13 kDa               | −0.52       | 6.9E-07   |
| 203491_s_at | CEP57       | centrosomal protein 57 kDa                                                  | −0.52       | 3.5E-06   |
| 235643_at | SAMD9L      | sterile alpha motif domain containing 9-like                                 | −0.52       | 1.7E-04   |
| 209662_at | CETN3       | centrin, EF-hand protein, 3 (CDC31 homolog, yeast)                          | −0.51       | 2.3E-04   |
| 212417_at | SCAMP1      | secretory carrier membrane protein 1                                        | −0.50       | 4.5E-04   |
| 217915_s_at | RSL24D1     | ribosomal L24 domain containing 1                                            | −0.49       | 2.6E-06   |
| 200598_s_at | HSP90B1     | heat shock protein 90 kDa beta (Grp94), member 1                            | −0.49       | 3.6E-07   |
| 242429_at | ZNF567      | zinc finger protein 567                                                      | −0.49       | 1.8E-05   |
| 232958_at | NA          | NA                                                                          | −0.48       | 5.6E-05   |
| 222326_at | NA          | NA                                                                          | −0.48       | 2.0E-05   |
| 200026_at | RPL34       | ribosomal protein L34                                                       | −0.47       | 3.1E-03   |
| 221765_at | UGCG        | UDP-glucose ceramide glucosyltransferase                                     | −0.47       | 3.1E-04   |
| 212794_s_at | KIAA1033    | KIAA1033                                                                    | −0.46       | 1.0E-06   |
| 200099_s_at | RPS3A       | ribosomal protein S3A                                                       | −0.46       | 2.2E-04   |
| 203153_at | IFIT1       | interferon-induced protein with tetratricopeptide repeats 1                | −0.46       | 2.9E-04   |
| 211968_s_at | HSP90AA1    | heat shock protein 90 kDa alpha (cytosolic), class A member 1               | −0.45       | 5.6E-06   |
| 226800_at | EFCA87      | EF-hand calcium binding domain 7                                             | −0.45       | 9.2E-09   |
| 225312_at | COMMD6      | COMMD domain containing 6                                                    | −0.44       | 6.1E-03   |
| 201699_at | PSMC6       | proteasome (prosome, macropain) 26S subunit, ATPase, 6                      | −0.44       | 4.4E-07   |
| 222848_at | CENPK       | centromere protein K                                                        | −0.44       | 2.4E-05   |
| 212587_s_at | PTPRC       | protein tyrosine phosphatase, receptor type, C                              | −0.43       | 1.7E-04   |
| 219239_s_at | ZNF654      | zinc finger protein 654                                                      | −0.43       | 3.0E-07   |
| 205849_s_at | UQCRB       | ubiquinol-cytochrome c reductase binding protein                            | −0.43       | 2.7E-03   |
| 214453_s_at | IFI44       | interferon-induced protein 44                                                | −0.43       | 6.8E-05   |
| 227152_at | C12orf35    | chromosome 12 open reading frame 35                                         | −0.43       | 7.2E-05   |
| 200061_s_at | RPS24       | ribosomal protein S24                                                       | −0.42       | 5.8E-03   |
| 205809_s_at | WASL        | Wiskott-Aldrich syndrome-like                                                | −0.42       | 4.0E-05   |
| 222616_s_at | USP16       | ubiquitin specific peptidase 16                                             | −0.42       | 6.0E-07   |
| 219356_s_at | CHMP5       | chromatin modifying protein 5                                                | −0.42       | 2.4E-05   |
| 244042_x_at | NA          | NA                                                                          | −0.41       | 4.0E-05   |
symptoms of allergic rhinitis according to the RQLQ scale. Pathway analysis suggested that allergic rhinitis patients treated with acupoint herbal plaster improved their balance of Th1-derived pro-inflammatory cytokines versus Th2-derived anti-inflammatory cytokines. Our results indicate that acupoint herbal plaster application diminished allergic inflammation by maintaining an appropriate equilibrium between Th1 and Th2 cells.

Conclusions
RQLQ and gene expression profiles indicated that patients with Ph-positive and -negative allergic rhinitis exhibit distinct physiological responses after receiving acupoint herbal plaster treatments. Gene expression levels were compared before and after acupoint herbal plaster application and in Ph-positive versus Ph-negative participants. In this preliminary study, we find that the IL-13 immune response via JAK-STAT signaling and interferon inflammation signaling were down- and upregulated, respectively, in the Ph-positive group. Further studies are required to verify these pathways in Ph-positive patients, and to determine the mechanism of such pathway dysregulation.

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Availability of data and materials
The datasets analyzed during the current study are presented in the manuscript or available on reasonable request from the corresponding author of this manuscript (Hen-Hong Chang) at Email: tcmchh55@gmail.com.

Authors’ contributions
SHS, LYS, and CHH conceived the study and designed the study protocol. SHS and LYS wrote the manuscript. CHH and TCN revised study protocols and wrote several sections of the manuscript. CHH and SHS coordinated and directed study implementation. LYS and TCN helped to develop study measures as well as data analysis and interpretation. All authors contributed to drafting the manuscript and have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interest.

Consent for publication
All authors have read and agreed to all the contents for publication.
Ethics approval and consent to participate
This human trial was approved by Chang Gung Memorial Hospital Institutional Review Board (IRB). All participants provided written consent forms.

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