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Gene Expression Profiles of HIV/AIDS Patients with *Qi-Yin* Deficiency and Dampness-Heat Retention

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Abstract

Objectives: Traditional Chinese Medicine (TCM) applied in the clinic as a complementary and alternative therapy has helped improve immunity and reduce side effects and symptomatic treatment in patients with HIV/AIDS. However, the mechanisms of TCM syndromes are not clear. Transcriptomics enables the study of such TCM syndromes.

Design: This study compared the messenger RNA (mRNA) expressions of healthy persons and patients with HIV/AIDS who had two common TCM syndromes, *qi-yin* deficiency and dampness-heat retention, to find the difference in HIV/AIDS with TCM syndromes.

Results: Comparison with healthy persons identified 113 mRNAs—41 enhanced and 72 decreased—in the *qi-yin* deficiency group. Additionally, 76 mRNAs were found in the dampness-heat retention group: 14 increased and 62 decreased. Functional genetic analysis of the mRNAs indicated that two TCM syndromes were correlated with cell apoptosis, immunoinflammatory responses, and lymphocyte activation. Differentially expressed mRNAs in the *qi-yin* deficiency group were obviously associated with cellular activity, communication, protein localization, cellular ion homeostasis, and regulation of cell motion, whereas mRNAs in the dampness-heat retention group were associated with sequence-specific DNA binding, cellular response to stress, and hemopoietic or lymphoid organ development.

Conclusions: These results suggest that the formation of different TCM syndromes in patients with HIV/AIDS were founded on biological transcriptomics, which reveal mechanisms of the formation of these syndromes in HIV/AIDS. Differentially expressed mRNAs in two TCM syndrome groups tended to normalize after TCM intervention, which indicates that TCM might remit symptoms by changing genetic expression.

Keywords: differentially expressed genes, AIDS, HIV, TCM syndromes, *qi-yin* deficiency syndrome, dampness-heat retention syndrome

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Introduction

PATHOGENESIS OF AIDS PARTLY depends on the ability of HIV to avoid host immunity; its ability to replicate in target cells, such as $CD4^+$ T cells; and induction of apoptosis of immune cells.[1] Although highly active anti-retroviral therapy can control viral load and incompletely restore immunity, it is associated with clinical drug resistance and side effects.[2,3] Alternative and complementary therapies have been positively verified.[4–6] Studies have shown that Traditional Chinese Medicine (TCM) has had some effects alone or when integrated with modern medicine in terms of reestablishing the immune system, reducing side effects, and personalized treatment.[7–9] TCM can be used as an alternative and complementary therapy for AIDS.[10] A precondition for effective clinical treatment with TCM has been the diagnosis of TCM syndromes in patients. Research on the molecular biological basis of TCM syndromes will provide a reference for diagnosis and evaluation of curative effect.[11,12]

The study of TCM syndromes in terms of transcriptomics is conducive to research on the molecular mechanisms of TCM syndromes and personalized treatment.[13–16] Transcriptomics explores genetic transcription and regulation in cells at the overall level of RNA expression and variability.[17,18] The interactions of differentially expressed genes might present different symptoms in patients, which corresponds to a characteristic of TCM: that is, TCM syndromes manifest timeliness and spatial awareness of a disease at a certain stage of development.

At present, some investigators have studied TCM syndromes in terms of transcriptomics.[19,20] Transcriptomics in patients with HIV/AIDS has also been studied.[21,22] However, gene expression profiles in HIV/AIDS with TCM syndromes has thus far not been explored. In addition, epidemiologic results have shown that the *qi-yin* deficiency syndrome[23–25] and dampness-heat retention[26,27] were the most common TCM syndromes in patients with HIV/AIDS in China.. Therefore, the present study selected patients with these two TCM syndromes with the objective of studying expression profiles and exploring the inherent foundation of HIV/AIDS in TCM patients at the molecular level.

Materials and Methods

General information

Forty-nine volunteers were included in this study. Among them, 29 patients with HIV/AIDS were from the Henan province of China: 12 with *qi-yin* deficiency syndrome and 17 with dampness-heat retention. Twenty healthy persons from the same region and same ethnic group were included as a control group to ensure that the age, sex, eating habits, and lifestyle of the control group were similar to those of the observation group. Baseline demographic characteristics, such as age and sex, did not significantly differ between groups (Tables 1 and 2).

TABLE 1. SEX DISTRIBUTION BETWEEN GROUPS

Group	*Men*	*Women*	*Total*
Case group (*n*)	17	12	29
Control group (*n*)	10	10	20

Chi-square = 0.356; $p = 0.551$.

TABLE 2. AGE DISTRIBUTION BETWEEN GROUPS

Group	*Participants* (n)	*Mean age (yr)*
Case group	29	47.95 ± 6.51
Control group	20	47.25 ± 8.13

Means are expressed with standard deviation. $t = 0.382$; $p = 0.703$.

The study was approved by the ''Prevention and Treatment of Serious Infectious Diseases Such as AIDS and Viral Hepatitis Prevention'' project management office of China. Each participant provided informed consent.

During the study, 5 mL of fasting anticoagulated blood was drawn from all participants in the morning; of this sample, 2 ml was used for transcriptomic research, and the other 3 mL was used for viral load and immune indices detection. This latter analysis provided a reference for basic conditions of the disease in participants included for transcriptomics research.

Inclusion criteria

Patients with HIV/AIDS included in the present study were aged 18–60 years and met the diagnostic criteria of AIDS as described in the *Diagnostic Guide of AIDS* recommended by the Ministry of Health, China, in 2008.

Criteria for TCM syndrome differentiation

TCM syndromes were differentiated in two steps. First, two professors made the initial diagnosis. Second, commonly used diagnostic scales for TCM syndromes in HIV/AIDS were used as diagnostic criteria. A TCM syndrome was diagnosed when the total points were greater than or equal to 20. The diagnostic scales were chosen according to the Science and Technology Prize of the China Association of TCM in 2012 (Epidemiologic Study and Diagnostic Scales of TCM Syndrome with HIV/AIDS) and the National Natural Science Foundation of China (Distribution Rule of TCM Syndromes in HIV/AIDS and Building and Verification of Syndrome Criteria, no. 90409004). These were considered for their diagnostic scales for *qi-yin* deficiency syndrome and dampness-heat retention syndrome as the diagnostic criteria[28] (Supplementary Tables S1 and S2; supplementary materials are available online at http://www.liebertpub.com/acm).

Exclusion criteria

The following were excluded from the study: (1) patients with AIDS in the middle or advanced stage of disease, with severe opportunistic infections; (2) patients with obnubilation, dementia, or other psychiatric/psychological disorders; (3) patients with severe organ disease or immunodeficiency disease that was induced by non-AIDS infections; and (4) patients who failed to provide informed consent for whatever reason.

Medicines and methods for intervention

Patients with HIV/AIDS who had corresponding TCM syndromes received an intervention with TCM granules (produced by Shenzhen San-Jiu Modern TCM Co., Ltd., in December 2010). The *qi-yin* deficiency group was treated with *Shengmai* decoction,[29] which consisted of 5 g of *Renshen* (*Panax ginseng*), 15 g of *Maidong* (*Ophiopogon japonicus*), and 10 g of Wuweizi (*Schisandra chinensis*). The dampness-

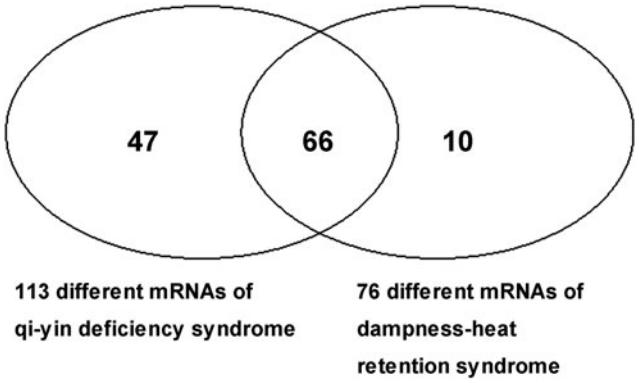

FIG. 1. Different messenger RNAs (mRNAs) of the two syndromes (*qi-yin* deficiency and dampness-heat retention).

heat retention group received *Sanren* decoction,[30] consisting 15 g of *Xingren* (*Amygdalus communis vas*), 20 g of *Yiyiren* (*Semen coicis*), 10 g of *Baikou* (cardamom), 12 g of *Jiangbanxia* (ginger processed *Pinellia ternata [Thunb.] Breit.*), 10 g of *Huashi* (talcum), 15 g of *Baizhu* (*Atractylodes macrocephala Koidz*), 15 g of *Houpu* (*Mangnolia officinalis Rehd. et Wils*), 6 g of *Tongcao* (*Tetrapanax papyriferus*), and 10 g of *Danzhuye* (*Herba Lophatheri*). Directions and dosage were provided to the patients and included one bag to be taken twice daily, with 200 mL of warm water added so that participants could drink the contents. The course of treatment lasted 3 months, repeated twice (two courses total). Blood samples were obtained before and after treatment.

Expression profile of the AFFX Human Genome U133 Plus 2.0 chips

Total RNA extraction reagent was applied to extract total RNA from fasting blood samples from the three groups. Appropriate RNA was detected by using a nucleic acid quantitative analyzer and agarose gel electrophoresis to visualize the bands. Expression profile of total RNA was tested by AFFX Human Genome U133 Plus 2.0 chips.

Data analysis

The differential signal points were analyzed and screened with the BRB-Array Tools 4.2 software program. With the GENE SET program, an expression-comparing tool that was included in the software, differential genes between groups were tested. BRB-ArrayTools, version 4.2, was used in cluster analysis on differentially expressed signals in the healthy and the disease groups. The information pathways of differentially expressed messenger RNAs (mRNAs) were analyzed online by using DAVID tools (http://david.abcc.ncifcrf.gov/), and the interactions between them were analyzed by using STRING (http://string.embl.de). SPSS software, version 21.0 (IBM Corp., Armonk, NY) was used for statistical analysis.

Results

Differential mRNAs in two syndrome groups

Differential signals were screened to determine their corresponding differential mRNAs according to the following criteria: (1) $p < 0.001$; (2) false discovery rate, $p < 0.05$; and (3) ratio of 0.5 or less and ratio greater than 2. Results showed that of the 113 differentially expressed mRNAs were found in the qi-yin deficiency group compared with the healthy group: 41 were upregulated (Supplementary Table S3) and 72 were downregulated (Supplementary Table S4). In addition, 76 differentially expressed mRNAs, 14 upregulated (Supplementary Table S5) and 62 downregulated (Supplementary Table S6) in the dampness-heat retention group, compared with the healthy group. With the exception of the shared differentially expressed mRNAs, 47

Table 3. Immunologic Indices Before Treatment per Study Group

Immunologic index	Qi-yin *deficiency pattern group*	*Dampness-heat retention pattern group*	*Control group*	F	p-*Value*
$CD4^+$ count (cells/mm^3)	349 ± 172[a]	372 ± 105[a]	880 ± 290	5.364	0.002
$CD8^+$ count (cell/mm^3)	641 ± 199	761 ± 245[a]	508 ± 253	3.866	0.013
$CD8^+$ $CD28^+/CD8^+$ (%)	39.19 ± 13.09	34.09 ± 8.04	39.24 ± 8.85	1.498	0.222
$CD8^+$ $CD38^+/CD8^+$ (%)	48.90 ± 16.40[a]	57.62 ± 20.20[a]	35.93 ± 10.16	7.234	<0.000
$CD4^+$ $CD45^+RA/CD4^+$ (%)	32.19 ± 12.07	26.91 ± 14.52[a]	38.55 ± 12.02	3.058	0.034
$CD4^+$ $CD45^+RO/CD4^+$ (%)	62.39 ± 10.21	70.37 ± 15.36	61.98 ± 11.73	1.860	0.144
$CD4^+$ $CD95^+/CD4^+$ (%)	45.59 ± 16.36	46.05 ± 16.35	38.13 ± 8.62	1.643	0.187
$CD4^+$ $CD28^+/CD4^+$ (%)	71.25 ± 16.08	61.05 ± 21.61[b]	77.61 ± 8.86	15.09	0.003
$CD4^+$ $CD25^+/CD4^+$ (%)	14.46 ± 8.90	8.71 ± 3.74	11.90 ± 4.41	2.463	0.069
$CD3^-CD16^+56^+$/Lym (%)	16.84 ± 10.72	16.64 ± 8.83	22.56 ± 11.19	1.986	0.096

Values expressed with a plus/minus sign are the mean ± standard deviation. SPSS 21.0 statistical software program was used to analyze relevant immune indices in different TCM syndromes. One-way analysis of variance was applied to analyze and compare the intergroup differences. To compare the differences in immune indices between groups, the least-squares difference method was selected for equal variances; the Dunnett T3 test was used for unequal variances. The results showed that when compared with values in the control group, the absolute value of $CD4^+$ T cells in both disease groups was reduced significantly, while the frequency of $CD8^+CD38^+$ T cells increased significantly ($p < 0.05$–0.01). Further, compared with the control group, the *qi-yin* deficiency group had a significant decrease in absolute values of $CD4^+$ and $CD8^+$ T cells ($p < 0.05$–0.01), while the dampness-heat retention group had a significant increase in CD8 T cells and reduced frequency of $CD4^+CD45RA^+$ and $CD4^+CD28^+$ dual-positive T cells ($p < 0.05$–0.01). There were no significant difference seen between groups in $CD8^+CD28^+$ and $CD4^+CD45RO^+$ T cells, $CD4^+CD95^+$, and $CD4^+CD25^+$ T cells or the $CD3^-CD16^+56^+$ T cells ($p > 0.05$).

[a]$p < 0.01$ compared with healthy controls.

[b]$p < 0.05$ compared with healthy controls.

Table 4. Predicted and Classified of Differential Genes in the Two Traditional Chinese Medicine Syndrome Groups

Number	*Parametric* p-*value*	t-*value*	*Fold-change*	*Gene symbol*
1	0.006712	−2.946	0.64	*DDX11L2*
2	0.006805	2.94	1.53	*PNP*
3	0.006365	2.968	1.61	*GPR18*
4	0.004938	3.072	2.42	*RPS26*
5	0.004692	3.093	2.26	*MS4A1*
6	0.003681	3.191	1.64	*BANK1*
7	0.002231	3.392	4.52	*XIST*

Differentially expressed genes in either group were predicted and classified according to the significance level of $p < 0.05$, fold change ≥ 1.5. The accuracy of compound covariate predictor was 86%; accuracy of diagonal linear discriminant analysis was 93%; accuracy of three nearest neighbors was 79%; accuracy of nearest centroid was 93%, accuracy of the support vector machines was 93%; and accuracy of the Bayesian compound covariate predictor was 96%.

mRNAs were specific to the *qi-yin* deficiency group and 10 were specific to the dampness-heat retention group (Fig. 1).

Prediction and classification of differentially expressed mRNAs

To verify the specificity of differentially expressed mRNAs in both syndrome groups, all differentially expressed genes in each group were predicted and classified according to the significance level of $p < 0.05$. In addition, fold-change of 1.5 or greater and seven genes, including *RPS26*, *MS4A1*, and *XIST* (Table 4), were screened. Differentially expressed mRNAs in both TCM syndrome groups could be distinguished, and the specific biological basis of the TCM syndromes could be verified.

Functional annotation clustering of differentially expressed mRNAs

The biological function of differentially expressed mRNAs in both TCM syndrome groups was analyzed by functional annotation clustering in DAVID tools. Some differentially expressed genes were correlated with apoptosis, response to material, immune response, chemotaxis,

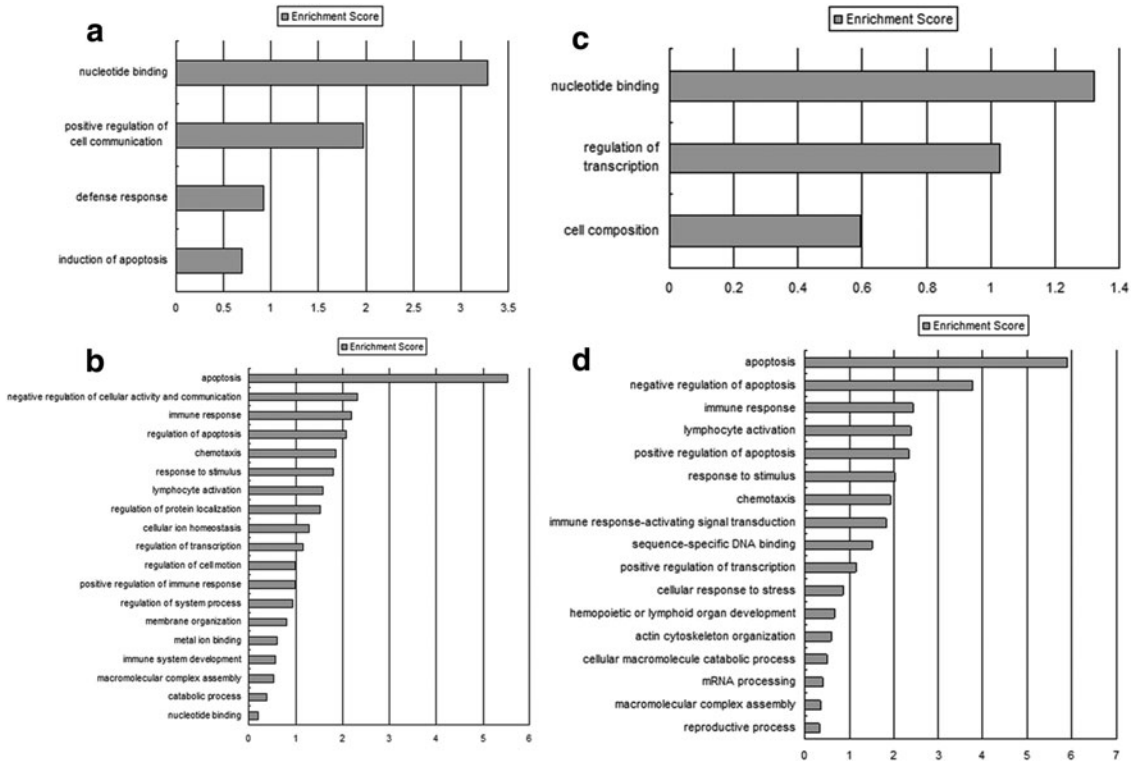

FIG. 2. Upregulated and downregulated mRNAs in both differential syndromes were analyzed by using DAVID tools. After the results of the functional annotation clustering were obtained, a bar chart was drawn with the enrichment score (y-axis) and function (x-axis). Differentially expressed genes that were relevant to the function with a greater enrichment score had a greater role in disease progression and syndrome formation in HIV. Functional annotation of upregulated and downregulated mRNAs in *qi-yin* deficiency syndrome was showed, respectively, in **(a)** and **(b)**. Functional annotation of upregulated and downregulated mRNAs was showed, respectively, in **(c)** and **(d)**.

Table 5. Signaling Pathways of Different Messenger RNAs of the *Qi-Yin* Deficiency Syndrome

Category	*Term*	*Count*	*%*	p-*Value*	*Gene symbol*
KEGG	hsa04062: Chemokine signaling pathway	9	0.87	1.93E-04	*CXCL1, GNAI3, PPBP, IL8, CXCR4, CCR2, CX3CR1, NFKBIA, GNG11*
KEGG	hsa04060: Cytokine-cytokine receptor interaction	9	0.87	0.001822	*CXCL1, IL1R2, TNFSF10, PPBP, IL8, CXCR4, CCR2, CX3CR1, IL1B*
KEGG	hsa04621: NOD-like receptor signaling pathway	5	0.48	0.002054	*CXCL1, IL8, NFKBIA, IL1B, TNFAIP3*
KEGG	hsa04210: Apoptosis	4	0.38	0.040987	*CFLAR, TNFSF10, NFKBIA, IL1B*
KEGG	hsa04620: Toll-like receptor signaling pathway	4	0.38	0.059209	*FOS, IL8, NFKBIA, IL1B*
KEGG	hsa04010: MAPK signaling pathway	6	0.58	0.084914	*FOS, IL1R2, DUSP1, RRAS2, JUND, IL1B*
BioCarta	h_nthiPathway: NFκB activation by nontypeable *Haemophilus influenzae*	4	0.38	0.003331	*DUSP1, IL8, NFKBIA, IL1B*
BioCarta	h_cd40Pathway: CD40L signaling pathway	3	0.29	0.013717	*DUSP1, NFKBIA, TNFAIP3*
BioCarta	h_tnfr2Pathway: TNFR2 signaling pathway	3	0.29	0.020021	*DUSP1, NFKBIA, TNFAIP3*
BioCarta	h_deathPathway: Induction of apoptosis through DR3 and DR4/5 death receptors	3	0.29	0.057829	*CFLAR, TNFSF10, NFKBIA*
BioCarta	h_il2rbPathway: IL-2 receptor β chain in T cell activation	3	0.29	0.068567	*FOS, CFLAR, SOCS3*

Signal pathways of differential genes in both syndromes were analyzed with DAVID tools (http://david. abcc.ncifcrf.gov/). Both syndromes had the same and specific signals. Both the differentially expressed genes and the statistical *p*-values of both Traditional Chinese Medicine syndromes were different in terms of the common signaling pathways.

KEGG, *Kyoto Encyclopedia of Genes and Genomes*; NOD, nucleotide oligomerization domain; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; TNFR2, tumor necrosis factor receptor 2; DR, death receptor; IL-2, interleukin-2.

Table 6. Signaling Pathways of Different Messenger RNAs of the Dampness-Heat Retention Syndrome

Category	*Term*	*Count*	*%*	p-*Value*	*Gene symbol*
KEGG	hsa04621: NOD-like receptor signaling pathway	6	0.84	4.18E-05	*CXCL1, IL8, NFKBIA, IL1B, RIPK2, TNFAIP3*
KEGG	hsa04620: Toll-like receptor signaling pathway	5	0.70	0.003855	*FOS, IL8, NFKBIA, IL1B, CCL4*
KEGG	hsa04062: Chemokine signaling pathway	6	0.84	0.006544	*CXCL1, IL8, CXCR4, CX3CR1, NFKBIA, CCL4*
KEGG	hsa04060: Cytokine-cytokine receptor interaction	6	0.84	0.025379	*CXCL1, IL8, CXCR4, CX3CR1, IL1B, CCL4*
KEGG	hsa04623: Cytosolic DNA-sensing pathway	3	0.42	0.048982	*NFKBIA, IL1B, CCL4*
KEGG	hsa05120: Epithelial cell signaling in *Helicobacter pylori* infection	3	0.42	0.071348	*CXCL1, IL8, NFKBIA*
BioCarta	h_nthiPathway: NFκB activation by nontypeable *H influenzae*	4	0.56	0.00239	*DUSP1, IL8, NFKBIA, IL1B*
BioCarta	h_Ccr5Pathway: Pertussis toxin–insensitive CCR5 signaling in macrophage	3	0.42	0.011031	*FOS, CXCR4, CCL4*
BioCarta	h_cd40Pathway: CD40L signaling pathway	3	0.42	0.011031	*DUSP1, NFKBIA, TNFAIP3*
BioCarta	h_tnfr2Pathway: TNFR2 signaling pathway	3	0.42	0.016146	*DUSP1, NFKBIA, TNFAIP3*
BioCarta	h_il2rbPathway: IL-2 receptor β chain in T cell activation	3	0.42	0.056103	*FOS, CFLAR, SOCS3*

Signal pathways of differential genes in both syndromes were analyzed with DAVID tools (http://david. abcc.ncifcrf.gov/). Both syndromes had the same and specific signals. Both the differentially expressed genes and the statistical *p*-values of both Traditional Chinese Medicine syndromes were different in terms of the common signaling pathways.

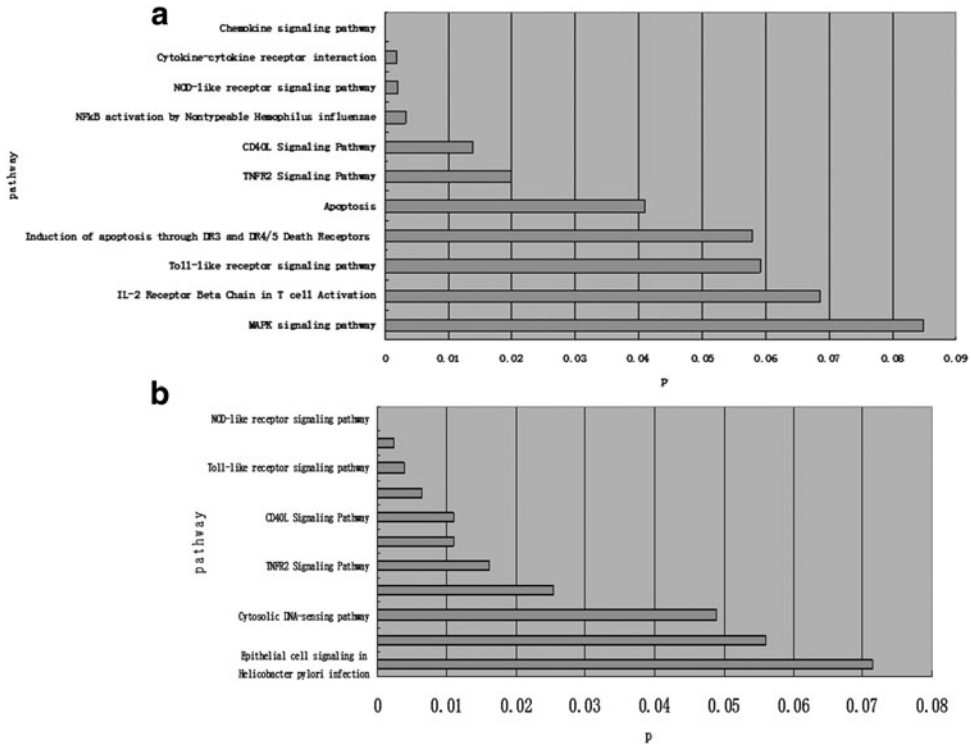

FIG. 3. Pathways of differentially expressed mRNAs in *qi-yin* deficiency syndrome **(a)** and dampness-heat retention syndrome **(b)** were obtained through DAVID tools. Bar charts were constructed, with the *p*-value displayed on the x-axis and the specific pathway on the y-axis. Pathway-related differential genes with lower *p*-values had a greater role in disease progression and syndrome formation seen in HIV/AIDS.

and lymphocyte activation (Supplementary Tables S7–S10). However, differential syndromes had different enrichment scores in the same annotation cluster.

Differentially expressed genes specific to both syndromes had differential functions. Functions specific to upregulated mRNAs in *qi-yin* deficiency syndrome were related to positive regulation of cell communication, induction of apoptosis, and other features (Fig. 2a). Downregulated mRNAs were obviously related to negative regulation of cellular activity and communication, regulation of protein localization, cellular ion homeostasis, and other features (Fig. 2b). Functions specific to the dampness-heat retention syndrome included the following: Upregulated mRNAs were correlated with regulation of transcription and cell composition (Fig. 2c), and downregulated mRNAs were involved in negative regulation of apoptosis, mRNA processing, sequence-specific DNA binding, cellular response to stress, hemopoietic or lymphoid organ development, and other features (Fig. 2d).

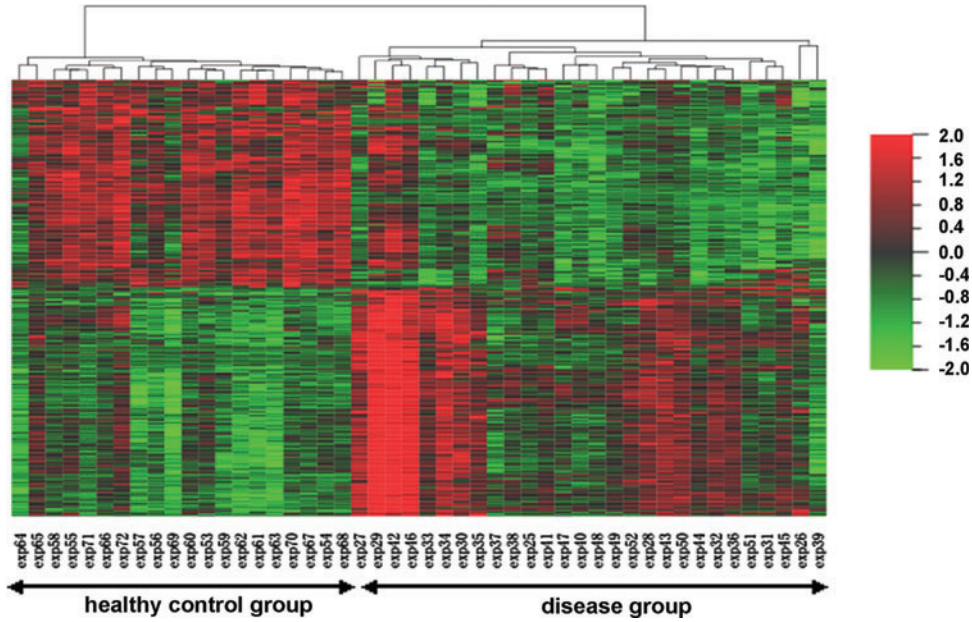

FIG. 4. With the BRB-Array Tools version 4.2 software, differentially expressed signals of the healthy control group and the syndrome groups were analyzed. Upregulated genes are shown in red and downregulated genes are displayed in green. From the image, it can be appreciated that the genetic expression in the healthy group and the disease groups was clearly different according to the color map. Partial differences in genetic expression for both syndrome groups could also be manifested in the cluster analysis image. Color images available online at www.liebertpub.com/acm

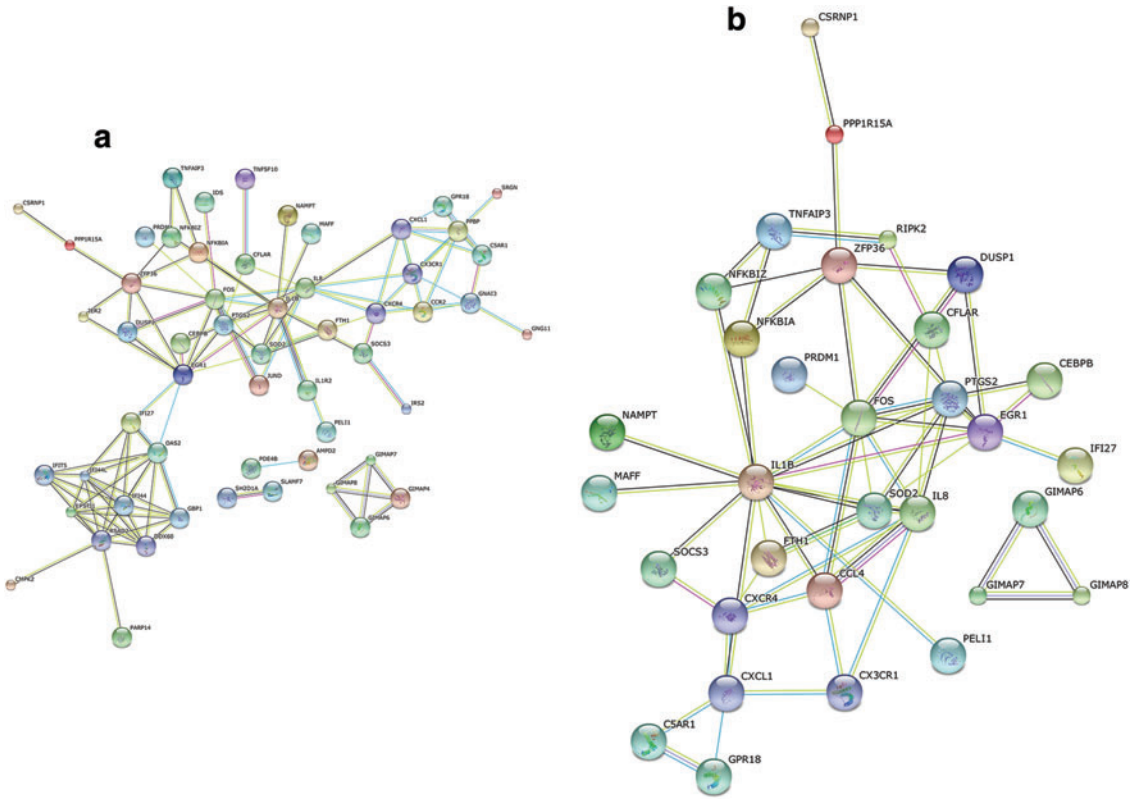

FIG. 5. Differential mRNAs of both syndromes were loaded into STRING. Required confidence (score) was 0.70, and interactors shown were no greater than 5. Differentially expressed mRNAs, which were closely related to each other, 56 mRNAs were found in the *qi-yin* deficiency syndrome **(a)**, while 32 mRNAs were found in the dampness-heat retention syndrome **(b)**. Lines in the diagram represent the relations between gene functions, whose intimacy level was represented by the length of the line. Color images available online at www.liebertpub.com/acm

Pathways of differentially expressed mRNAs

To further grasp the mechanism of differentially expressed genes in TCM syndromes, DAVID tools were applied to analyze the pathways of these genes. Both syndromes had common pathways and specific pathways (Tables 5 and 6; Fig. 3a and 3b).

Cluster analysis

Differentially expressed signals of the healthy group and both of the syndrome groups were analyzed with BRB-Array Tools, version 4.2. According to the cluster analysis of the genetic expression levels, both the healthy group and the disease groups can be distinguished in color, which indicated that the healthy group and the disease groups had different genetic expression profiles. Both syndrome groups could also be partly differentiated, which showed that there were both similar the specific differentially expressed mRNAs seen between different syndromes (Fig. 4).

Interaction between differentially expressed genes

Differentially expressed mRNAs of both syndromes were input into STRING in order to intuitively study the correlation between them. The required confidence (score) was 0.70, and the interactors shown yielded a value that was no greater than 5. Differentially expressed mRNAs, which were closely related to each other, were found in the *qi-yin* deficiency syndrome group, making up a relationship chart with 56 nodal points and a side-length of 112 (Fig. 5a). According to the same standards, 32 differentially expressed mRNAs, which were closely related to each other, were found in the dampness-heat retention syndrome group, forming a relationship chart with 32 nodal points and a side-length of 54 (Fig. 5b).

Table 7. Scores of the Traditional Chinese Medicine Syndrome Before and After Treatment

Group	*Participants* (n)	*Scores before TCM treatment*	*Scores after TCM treatment*	t	p-*Value*
Qi-yin deficiency syndrome	12	32.42 ± 10.30	13.18 ± 2.56	4.859	0.0001
Dampness-heat retention syndrome	17	29.47 ± 6.51	10.46 ± 6.54	7.909	0.0001

Values expressed with a plus/minus sign are means ± standard deviation.
TCM, Traditional Chinese Medicine.

Table 8. Immune Indices Before and After TCM Treatment

	Qi-yin *deficiency syndrome*		*Dampness-heat retention syndrome*	
Immune index	*Before TCM treatment*	*After TCM treatment*	*Before TCM treatment*	*After TCM treatment*
$CD4^+$ count (cells/mm^3)	349 ± 172	389 ± 264	372 ± 105	386 ± 152
$CD8^+$ count (cells/mm^3)	641 ± 199	985 ± 477[a]	761 ± 245	896 ± 274
$CD8^+$ $CD28^+/CD8^+$ (%)	39.19 ± 13.09	62.17 ± 16.22[b]	34.09 ± 8.04	57.85 ± 12.98[b]
$CD8^+$ $CD38^+/CD8^+$ (%)	48.90 ± 16.40	38.29 ± 14.07	57.62 ± 20.20	40.88 ± 13.36[b]
$CD4^+$ $CD45^+RA/CD4^+$ (%)	32.19 ± 12.07	31.87 ± 9.73	26.91 ± 14.52	22.68 ± 12.32
$CD4^+$ $CD45^+RO/CD4^+$ (%)	62.39 ± 10.21	57.10 ± 16.03	70.37 ± 15.36	59.78 ± 19.51
$CD4^+$ $CD95^+/CD4^+$ (%)	45.59 ± 16.36	61.25 ± 20.25[a]	46.05 ± 16.35	60.59 ± 18.56[a]
$CD4^+$ $CD28^+/CD4^+$ (%)	71.25 ± 16.08	53.79 ± 23.90	61.05 ± 21.61	64.36 ± 22.07
$CD4^+$ $CD25^+/CD4^+$ (%)	14.46 ± 8.90	17.57 ± 11.38	8.71 ± 3.74	11.06 ± 4.68
$CD3^-CD16^+56^+$/Lym (%)	16.84 ± 10.72	17.84 ± 10.85	16.64 ± 8.83	17.71 ± 8.02

The differences in the immune indices before and after TCM treatment were analyzed and compared. After receipt of medicine, $CD8^+CD28^+$ T cells and $CD4^+CD95^+$ T cells increased significantly in both syndrome groups ($p < 0.05$–0.01). The absolute value of $CD8^+$ T cells increased significantly in the *qi-yin* deficiency syndrome group ($p < 0.05$) and the $CD8^+CD38^+$ T cells were reduced sharply in the dampness-heat retention syndrome group ($p < 0.01$). There were no significant changes in other indices ($p > 0.05$).

[a]$p < 0.05$ for comparison of before and after TCM treatment.

[b]$p < 0.01$ for comparison of before and after TCM treatment.

Changes in mRNA expression in both syndromes after TCM treatment

To further grasp the roles of differential genes in TCM treatment, two TCM interventions were applied to treat patients with HIV/AIDS and the corresponding syndromes. Clinical symptoms total scores were reduced after treatment in two pattern groups ($p < 0.05$; Table 7), and immunologic indexes changed after treatment ($p < 0.05$; Table 8). These results can objectively prove the reliability of the TCM curative effect.

Comparison of genetic expression in the healthy control group before and after treatment showed that 18 mRNAs in patients with HIV/AIDS normalized; this involved 15 pathways that were mainly related to cellular apoptosis and inflammation (Tables 9 and 10). Moreover, 35 mRNAs in the *qi-yin* deficiency group normalized and involved eight pathways (Tables 11 and 12). In addition, 25 mRNAs in the dampness-heat retention syndrome that involved 17 pathways normalized (Tables 13 and 14). Through the prognosis of genes, the material basis of transcriptomic analysis in the *qi-yin* deficiency syndrome and the dampness-heat retention syndrome could be initially verified.

After the intervention, the normalized genes in both syndrome groups were obtained and separately applied into hierarchical clustering analysis with the genes in the patient groups and the healthy control group. Results showed that compared with the expression before treatment, the expression of genes in both syndrome groups after treatment was close to that shown for the healthy control group. In addition, even some genes in the *qi-yin* deficiency syndrome were misclassified into the healthy control group after treatment (Fig. 6a and 6b).

Table 9. Eighteen Genetic Expressions Close to the Healthy Control Group in HIV/AIDS Patients, Normalized

Probe set ID	*Gene symbol*	*After vs. healthy*	*Before vs. healthy*	*After vs. before*
230333_at	–	0.6658	0.314186	2.0196
236495_at	–	0.6002	0.260687	2.2371
243296_at	*NAMPT*	0.5341	0.237821	2.1741
243509_at	–	0.5337	0.236277	2.1398
205681_at	*BCL2A1*	0.4042	0.22675	1.7128
201502_s_at	*NFKBIA*	0.3995	0.159954	2.3892
223217_s_at	*NFKBIZ*	0.3825	0.180093	1.9869
223218_s_at	*NFKBIZ*	0.3218	0.194865	1.5828
204748_at	*PTGS2*	0.3175	0.113928	2.6392
213524_s_at	*G0S2*	0.1948	0.022876	7.1192
202859_x_at	*IL8*	0.1694	0.035374	4.6883
212130_x_at	*EIF1*	0.6739	0.435402	1.5164
212227_x_at	*EIF1*	0.6885	0.446181	1.5024
230529_at	*HECA*	0.7672	0.493156	1.537
201531_at	*ZFP36*	0.7496	0.462924	1.6302
239757_at	*ZFAND6*	0.7987	0.468069	1.6562
204285_s_at	*PMAIP1*	0.7278	0.385722	1.8355
212341_at	*YIPF6*	1.245	0.64449	1.8954

Table 10. Pathway of Genetic Expressions Close to the Healthy Control Group in HIV/AIDS Patients, Normalized

Pathway	*Count*	p-*Value*	q-*Value*	*Gene symbol*
Epithelial cell signaling in *H pylori* infection	2	1.50E-04	9.09E-05	*NFKBIA, IL8*
Small-cell lung cancer	2	2.27E-04	9.09E-05	*NFKBIA, PTGS2*
Toll-like receptor signaling pathway	2	3.19E-04	1.06E-04	*NFKBIA, IL8*
Nicotinate and nicotinamide metabolism	1	0.00627	2.70E-04	*NAMPT*
Bladder cancer	1	0.010949	4.13E-04	*IL8*
Arachidonic acid metabolism	1	0.014833	5.20E-04	*PTGS2*
Adipocytokine signaling pathway	1	0.017414	6.00E-04	*NFKBIA*
p53 signaling pathway	1	0.017929	6.03E-04	*PMAIP1*
B cell receptor signaling pathway	1	0.019474	6.25E-04	*NFKBIA*
Chronic myeloid leukemia	1	0.019474	6.25E-04	*NFKBIA*
VEGF signaling pathway	1	0.019732	6.25E-04	*PTGS2*
Prostate cancer	1	0.022814	6.89E-04	*NFKBIA*
Apoptosis	1	0.023071	6.89E-04	*NFKBIA*
T cell receptor signaling pathway	1	0.028188	7.27E-04	*NFKBIA*
Cytokine-cytokine receptor interaction	1	0.066526	0.001401	*IL8*

HIV/AIDS patients with both syndromes were considered as disease groups. The genetic expression profiles seen before and after drug intervention were compared. After intervention, the degree of expression of 18 differentially expressed genes progressed toward the healthy control group. Through analysis of signaling pathways, 15 pathways, such as cellular apoptosis and inflammation, were related to normalized genes. When $p < 0.05$, there were 14 signaling pathways related to normalized genes.

VEGF, vascular endothelial growth factor.

Table 11. Thirty-five Genetic Expressions Close to the Healthy Control Group in the *Qi-Yin* Deficiency Group

Probe set ID	*Gene symbol*	*After vs. healthy*	*Before vs. healthy*	*After vs. before*
206765_at	*KCNJ2*	2.1083	2.994986	0.6208
242625_at	*RSAD2*	2.0502	4.478901	0.6183
202270_at	*GBP1*	1.574	2.77847	0.6169
214329_x_at	*TNFSF10*	1.5426	2.806688	0.6211
228071_at	*GIMAP7*	1.5416	2.744963	0.566
203761_at	*SLA*	1.2939	2.065447	0.6
219243_at	*GIMAP4*	1.362	2.025492	0.6612
209112_at	*CDKN1B*	0.7699	1.533911	0.49
203455_s_at	*SAT1*	0.6496	0.414196	1.7452
210592_s_at	*SAT1*	0.6478	0.395959	1.7694
236495_at	–	0.6052	0.204204	2.1291
201041_s_at	*DUSP1*	0.5901	0.178339	3.3486
225557_at	*CSRNP1*	0.5773	0.399585	1.5136
211998_at	*H3F3B*	0.5082	0.308164	1.8047
214211_at	*FTH1*	0.4948	0.303608	1.562
243296_at	*NAMPT*	0.4463	0.22214	2.0051
243509_at	–	0.4416	0.202034	2.2336
205681_at	*BCL2A1*	0.3947	0.217273	1.9054
201502_s_at	*NFKBIA*	0.3884	0.160713	2.451
204748_at	*PTGS2*	0.3686	0.102077	3.5925
204470_at	*CXCL1*	0.3623	0.208837	1.5576
223217_s_at	*NFKBIZ*	0.3587	0.169925	2.1394
223218_s_at	*NFKBIZ*	0.3464	0.189468	2.0542
205114_s_at	*CCL3*	0.2607	0.162641	1.9618
213524_s_at	*G0S2*	0.224	0.017729	12.0734
202859_x_at	*IL8*	0.166	0.030291	5.7922
201473_at	*JUNB*	0.8657	0.489024	1.7308
220046_s_at	*CCNL1*	0.7697	0.502441	1.7463
230529_at	*HECA*	0.7359	0.446418	1.6649
202081_at	*IER2*	0.6942	0.469971	1.5566
201531_at	*ZFP36*	0.7612	0.411515	1.8872
239757_at	*ZFAND6*	0.7993	0.42851	1.9121
204285_s_at	*PMAIP1*	0.7395	0.404904	1.9356
1555411_a_at	*CCNL1*	0.7678	0.486916	1.5989
200844_s_at	*PRDX6*	1.5956	0.638738	1.9763

Table 12. Pathway of Genetic Expression Close to the Healthy Control Group in the *Qi-Yin* Deficiency Group

Pathway	*Count*	p-*Value*	q-*Value*	*Gene symbol*
Epithelial cell signaling in *H pylori* infection	3	1.64E-05	4.11E-06	*NFKBIA, CXCL1, IL8*
Small-cell lung cancer	3	3.05E-05	6.03E-06	*CDKN1B, NFKBIA, PTGS2*
Cytokine-cytokine receptor interaction	4	3.32E-05	6.03E-06	*TNFSF10, CXCL1, CCL3, IL8*
Toll-like receptor signaling pathway	3	5.07E-05	6.76E-06	*NFKBIA, CCL3, IL8*
Systemic lupus erythematosus	3	1.41E-04	1.28E-05	*H3F3B, LOC644914, H3F3A*
Chronic myeloid leukemia	2	0.001271	7.48E-05	*CDKN1B, NFKBIA*
Prostate cancer	2	0.001743	9.90E-05	*CDKN1B, NFKBIA*
Apoptosis	2	0.001783	9.90E-05	*TNFSF10, NFKBIA*

Gene expression in HIV/AIDS patients with the *qi-yin deficiency* syndrome before and after intervention was compared. After intervention, 35 differentially expressed genes that involved eight signaling pathways progressed toward the healthy control group ($p < 0.05$). Compared with the results seen in the dampness-heat retention group, both syndromes had both similar and different pathways, and normalized genes that were present in both syndromes were not all the same.

Discussion

TCM syndrome is an overall manifestation of a disease at a certain stage of development. It may have some correlation with differentially expressed mRNAs in the body. Transcriptomics applies chip technology to explore relevant genetic expression profiles of differential syndromes in different individuals and provides new ideas to help explain the essence of TCM syndromes in a microscopic view.

In the present study, there were shared and specific differentially expressed mRNAs in the healthy control group and both syndrome groups. Shared mRNAs may be related to the progression of HIV/AIDS, while specific genes may play a significant role in the formation of TCM syndromes in HIV/AIDS. Massive abnormal apoptosis of T cells plays a critical role in HIV infection and immunopathogenesis of AIDS. HIV evasion and induction of lymphocyte apoptosis are the main reasons for the induction of AIDS progression.[31–33]

In the present study, differentially expressed mRNAs were found in two TCM syndrome groups; *BCL2A1, IL1B, TNFAIP3, PTGS2,* and *NFKBIA* were related to cellular apoptosis and regulation of apoptosis. Nuclear factor-κB (NF-κB) participates in the proliferation and apoptosis of lymphocytes,[34,35] while *BCL2A1* is a target gene in the inflammatory response that is induced by transcribed NF-κB and plays an important role in activation and survival of lymphocytes.[36,37] Family members of BCL can help B cells in patients with HIV/AIDS evade elimination by Fas-mediated

Table 13. Twenty-five Genetic Expression Close to the Healthy Control Group in the Dampness-Heat Retention Group

Probe set ID	*Gene symbol*	*After vs. healthy*	*Before vs. healthy*	*After vs. before*
213524_s_at	*G0S2*	0.2021	0.024449	6.1832
202859_x_at	*IL8*	0.1932	0.036015	4.7861
201502_s_at	*NFKBIA*	0.4464	0.162281	2.6321
243296_at	*NAMPT*	0.6436	0.230006	2.5917
204748_at	*PTGS2*	0.3259	0.115045	2.5699
223217_s_at	*NFKBIZ*	0.4537	0.176858	2.2493
243509_at	–	0.619	0.253299	2.2321
201041_s_at	*DUSP1*	0.6134	0.280119	2.1092
205681_at	*BCL2A1*	0.4517	0.208313	2.0323
223218_s_at	*NFKBIZ*	0.4019	0.190931	1.7416
230333_at	–	0.6397	0.310985	1.6559
211998_at	*H3F3B*	0.5456	0.317318	1.6488
210592_s_at	*SAT1*	0.63	0.393698	1.5412
236495_at	–	0.6729	0.258998	2.8924
232304_at	*PELI1*	0.7174	0.396169	1.8565
212341_at	*YIPF6*	1.3195	0.663453	1.8313
230529_at	*HECA*	0.8578	0.520437	1.6856
201531_at	*ZFP36*	0.8175	0.490776	1.5987
241133_at	*TRBV27*	0.7664	0.485686	1.592
204115_at	*GNG11*	0.8588	0.565384	1.5907
212130_x_at	*EIF1*	0.6802	0.422807	1.5785
204838_s_at	*MLH3*	0.7652	0.472423	1.5615
202021_x_at	*EIF1*	0.6741	0.43391	1.5512
212227_x_at	*EIF1*	0.6856	0.436141	1.5406
217783_s_at	*YPEL5*	0.7626	0.486319	1.5082

Table 14. Pathways of Genetic Expression Close to the Healthy Control Group in the Dampness-Heat Retention Group

Pathway	*Count*	p-*Value*	q-*Value*	*Gene symbol*
Systemic lupus erythematosus	3	3.34E-05	9.53E-06	*H3F3B, LOC644914, H3F3A*
Epithelial cell signaling in *H pylori* infection	2	4.30E-04	6.61E-05	*IL8, NFKBIA*
Small cell lung cancer	2	6.48E-04	9.25E-05	*NFKBIA, PTGS2*
Toll-like receptor signaling pathway	2	9.09E-04	1.21E-04	*IL8, NFKBIA*
Mismatch repair	1	0.009997	3.69E-04	*MLH3*
Nicotinate and nicotinamide metabolism	1	0.010429	3.69E-04	*NAMPT*
Urea cycle and metabolism of amino groups	1	0.012157	4.12E-04	*SAT1*
Bladder cancer	1	0.018183	5.77E-04	*IL8*
Arachidonic acid metabolism	1	0.024601	7.34E-04	*PTGS2*
Adipocytokine signaling pathway	1	0.028857	8.49E-04	*NFKBIA*
B cell receptor signaling pathway	1	0.032249	9.07E-04	*NFKBIA*
Chronic myeloid leukemia	1	0.032249	9.07E-04	*NFKBIA*
VEGF signaling pathway	1	0.032672	9.07E-04	*PTGS2*
Prostate cancer	1	0.037737	9.91E-04	*NFKBIA*
Apoptosis	1	0.038158	9.91E-04	*NFKBIA*
T cell receptor signaling pathway	1	0.046541	0.001029	*NFKBIA*
Cytokine-cytokine receptor interaction	1	0.108409	0.001792	*IL8*
MAPK signaling pathway	1	0.113486	0.00183	*DUSP1*

Gene expression in HIV/AIDS patients with the dampness-heat retention syndrome before and after intervention was compared. After intervention, 25 differentially expressed genes that involved 16 signaling pathways progressed toward the healthy control group ($p < 0.05$). Compared with the results seen in the *qi-yin* deficiency syndrome group, both syndromes had both similar and different pathways, and normalized genes that were present in both syndromes were not all the same.

cell death.[38,39] In addition, *NFKBIA* (NF-κB inhibitor α) encodes a protein that blocks NF-κB.[40] After TCM intervention, *BCL2A1, NFKBIA,* and *PTGS2* tended to normalize. Different expression of these genes plays a certain role in apoptosis of immune cells in patients with HIV/AIDS.[41] Prognosis after intervention initially verified that therapeutics might be effective in situations where altered gene expression is evident.

As the research on the relationships of HIV infection with chemokines and immune response has deepened, some chemokine receptors have been shown to be related to HIV-1 infection.[42–45] When infected with HIV, chemotactic factors such as CXCR4 and CCR5 can cause apoptosis upon interacting with the envelope glycoprotein gp120 of HIV-1, permitting entry of the virus into viable T lymphocytes.[46] In the present study, differentially expressed genes relevant to chemokine and immune response pathways, such as *IL1B*, *IL8*, and *NFKBIA,* were detected. Secreted by many kinds of cells in the inflammatory response, interleukin (IL)-8 can induce inflammatory responses of chemokines and participate in neutrophil activation.[47,48] The content of IL-8 and tumor necrosis factor-α increased when mononuclear cells of healthy individuals were infected with HIV in the early stages of disease.[48] The IL-1 family can regulate the inflammatory response, while polymorphisms in the IL-1B family were significantly associated with HIV-1 infection.[50]

In the present study, unlike in the early stages of monocyte infection, downregulated expression of IL-8 and IL-1B were related to dysfunction of immune cells caused by long-term infection with HIV. In addition, *NFKBIA* is the regulatory hub for genetic transcription related to many immune-mediated inflammatory processes through the NF-κB pathway.[51,52] After treatment, *IL8*, *IL1B*, and *NFKBIA* became normalized, indicating that therapeutics can influence the expression of these genes.

AIDS is a chronic immune deficiency disorder, and *qi-yin* deficiency syndrome is one of the common deficiency syndromes in AIDS. Different TCM syndromes have different pathologies, and differentially expressed genes in both syndromes have their own specificities.

Through comparison of differentially expressed mRNA in both syndromes, the enrichment score of apoptosis for mRNA downregulated in the *qi-yin* deficiency syndrome group was lower than the score in the dampness-heat retention syndrome group. In addition, the mRNA that was involved in apoptosis and upregulated in the *qi-yin* deficiency syndrome group was found through functional annotation clustering. CCR2, CD274, and TNFSF10 were expressed differentially in the *qi-yin* deficiency syndrome group but showed no obvious changes in the dampness-heat retention syndrome group.

It was believed that polymorphism of CCR2 was related to host immunity, sensitivity to HIV-1 infection, and progression of disease.[53] The interactions between CD274 (B7 H1) and programmed cell death factor 1 can inhibit immune responses of T cells by inhibiting the proliferation of T cells and the generation of cell factor.[54,55] The enrichment score for negative regulation of cellular activity and communication downregulated in the *qi-yin* deficiency syndrome group was greater than that in the dampness-heat retention syndrome group. The pathway of the differentially expressed mRNAs in the two different syndromes showed that differential expression related to chemokines and cellular factors was more obvious in the *qi-yin* deficiency group.

The results of immune functional analyses showed that compared with the dampness-heat retention group and healthy control group, the absolute value of $CD4^+$ and $CD8^+$

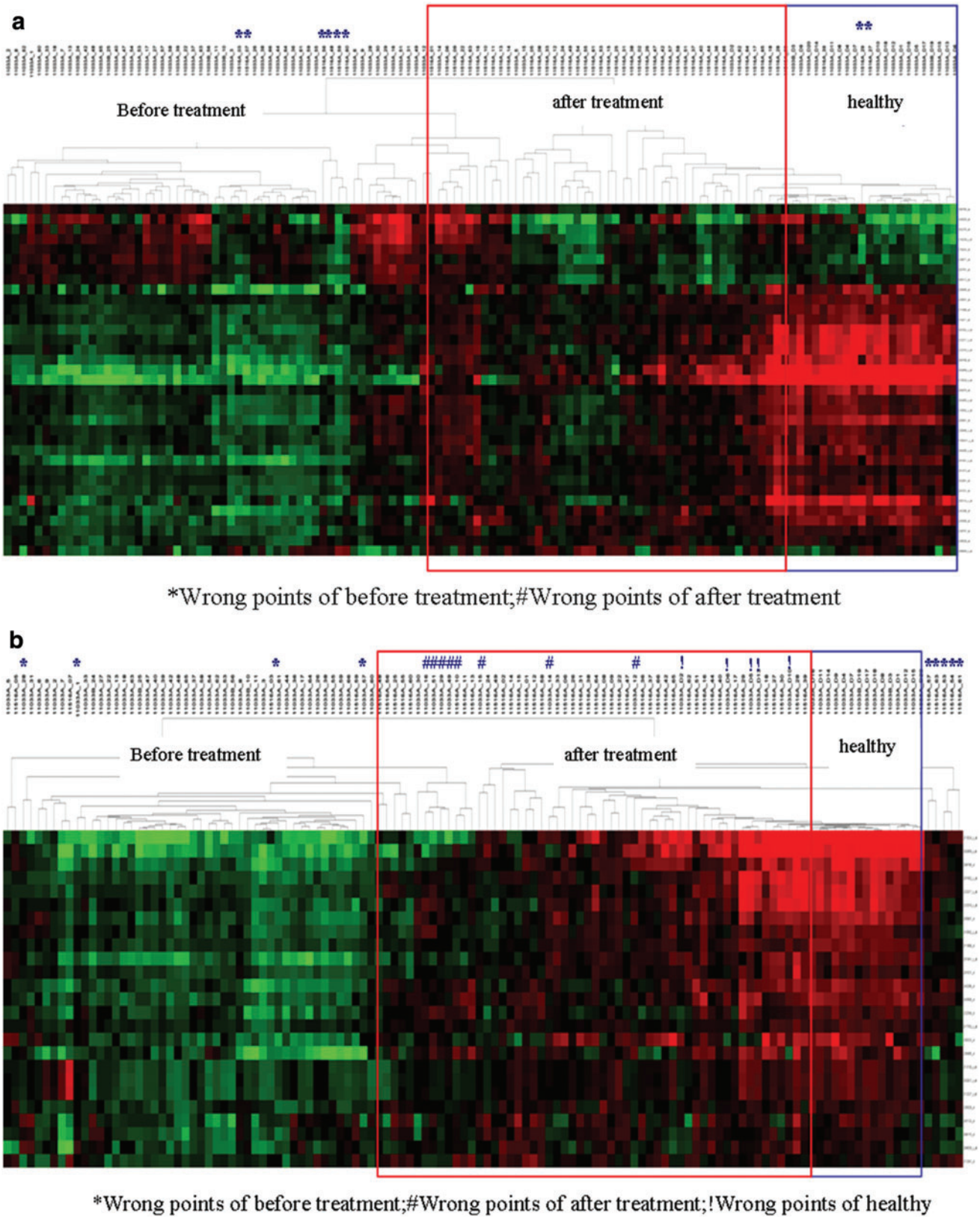

FIG. 6. With BRB-Array Tools version 4.2, mRNAs in both syndromes before and after treatment and in the healthy group were analyzed by hierarchical clustering. *Red* represents the increase in genetic expression levels, while *green* represents a decrease in expression. In the *qi-yin* deficiency syndrome **(a)**, some samples were misclassified into the prior-treatment disease group and the normal control group by following the intervention. Misclassification into prior treatment, post-treatment, and normal samples was also found in the dampness-heat retention group **(b)**. After treatment, both syndrome groups were closer to the control group, and the expression levels of differentially expressed genes was normalized. Color images available online at www.liebertpub.com/acm

T cells was smaller in the *qi-yin* deficiency group. This finding indicated that patients with HIV/AIDS and *qi-yin* deficiency had more severe immune damage. According to other studies in the field, inflammation and genetic polymorphism of cellular factors were related to fatigue in adults with HIV/AIDS.[56] The pathological mechanism still requires further study and remains largely unresolved.

The dampness-heat retention syndrome is one of the commonly seen excess syndromes in AIDS. Some scholars found that the activity of cellular factors, apoptosis and proliferation regulation had changed in patients with hepatitis and those with gastritis who had dampness-heat retention.[57,58] Downregulated genes that were related to cell composition, cell apoptosis, and lymphocyte activation and upregulated genes that were related to cell composition were detected in the dampness-heat retention group. These genes may be associated with dampness-heat retention symptoms, such as diarrhea and bitter and sticky mouth. It was found that 36%–56% of hospitalized patients with AIDS had hyponatremia and 16%–24% of patients had hyperkalemia.[59] In the dampness-heat retention patients, upregulated genes were involved in potassium ion transport

By classification and prediction of differentially expressed genes in both syndrome groups, seven mRNAs were screened. The findings showed that these seven mRNAs may be the biological basis of both syndromes; this result was significant in the context of the differentiation of TCM syndromes in terms of genetic expression. The interaction diagram of differentially expressed mRNAs of both syndromes showed that these differentially expressed mRNAs were related to each other, forming a complex informational network. The correlations of differential mRNAs also differed between both syndromes, which indicated that these genes played an important role in patients with AIDS presenting with different syndromes.

Conclusions

Through testing the expression profiles of total RNA in the peripheral blood of patients with HIV/AIDS who had *qi-yin* deficiency and dampness-heat retention, the present study predicted in an exploratory way that an inherent biological basis might have been responsible for the TCM syndromes in these patients. Further, the functions of differentially expressed mRNAs and their pathways might also be related to the formation of two common syndromes in patients with HIV/AIDS, which provides a useful reference point for further research on the inherent complexities of TCM syndromes in AIDS.

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Author Disclosure Statement

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