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ORIGINAL RESEARCH

# Altered Gene Expression in Acne Vulgaris Patients Treated by Oral Isotretinoin: A Preliminary Study

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**Background/Objective:** The role of gene expression changes in acne patients treated by oral isotretinoin (ISO) and in influencing the ISO therapeutic effects is still unclear. In this study, we investigated the gene profiles of patients with severe acne who responded variously to ISO therapy.

**Methods:** The peripheral blood of 113 acne vulgaris patients (Pillsbury IV grade) was collected before treatment. After 8 weeks of oral ISO, nine acne patients were selected and divided into the following groups. A: effectively treated by ISO, group B: ineffectively treated by ISO, group C: ISO-induced acne flare-up, and 3 healthy subjects were included as control group D. The peripheral blood of patients pre- and post-treatment was subjected to high-throughput RNA sequencing technology and bioinformatics analysis of the separate groups (n = 3). The candidate genes were validated by qRT-PCR.

**Results:** Comparing pre- and post-oral ISO treatment, gene expression was changed as 39 genes in ISO-effective group, 345 genes in ISO-ineffective group, and 57 genes in ISO-induced acne flare-up group. Comparing the ISO-induced acne flare-up group with healthy control subjects revealed 34 upregulated genes and 23 downregulated genes, while comparing the ISO-induced acne flare-up group with ISO-ineffective patients identified 1835 changed genes. Expression of *GATA2* (2.73 fold, P=0.024512), *C4BPA* (35.87 folds, P=0.038073), and *CCR5* (2.48 folds, P=0.004681) increased in the ISO-induced acne flare-up patients. Meanwhile, the expression of *DEFA3* (0.18 fold, P=0.041934), *ELANE* (0.14 fold, P=0.030767), *MMP9* (0.41 fold, P=0.013383), and *RPS4Y1* (0.00018 fold, P=0.000986) decreased when compared with ISO-ineffective patients.

**Conclusion:** Oral ISO treatment could temporarily alter gene expression in acne patients. ISO therapeutic mechanisms were involved, not only in regulating the inflammatory reaction but also in the process of DNA repair. *GATA2, C4BPA, CCR5, DEFA3, ELANE, MMP9*, and *RPS4Y1* might be susceptible to genes that could participate in the ISO-induced aggravation of acne.

**Keywords:** acne vulgaris, isotretinoin, treatment, gene profile, mechanisms

## Introduction

Acne is a common chronic skin disease with a multifactorial etiology and pathogenesis. Recent studies have revealed that genetic factors play a role in the pathogenesis of acne vulgaris.[1–5] Transcriptional profiling has successfully been used to investigate the pathogenesis of various diseases like psoriasis,[6] atopic dermatitis,[7] and so forth. RNA sequencing, combined with bioinformatics has become a powerful tool for the detection of gene expression changes and has been useful for screening out specific genes and pathways.[8]

Oral isotretinoin (ISO) is recommended for the treatment of severe nodular acne and, by normalizing follicular desquamation, decreasing sebum secretion, inhibiting the growth of P. acnes, and exerting anti-inflammatory effects, it targets all four

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components involved in the development of acne.[9] Studies have revealed that oral ISO could change the expression of some genes and that it might act via anti-inflammation and sebocyte apoptosis effects[10] through increasing the expression of the FoxO1 gene[11] and cell cycle inhibitor p21.[12] Furthermore, Nelson et al used microarray and reported the upregulation of extracellular matrix proteins encoding genes and the downregulation of numerous genes encoding lipid metabolizing enzymes in the skin of acne patients treated with ISO for 8 weeks.[13] These previous findings identified that gene expression could be affected by acne treatment medicines.

However, changes in the gene profiles of acne patients undergoing ISO therapy and whether or not genetic factors could influence ISO therapeutic effects remain still unclear. In this study, we sequenced and compared the gene profiles of acne patients who responded variously to ISO therapy and identified the potential physiological roles.

# Patients and Methods

## Ethics Statement

The human studies were approved by the Clinical Research Ethics Committee at the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China ([2018]02–194-01). The consent procedure was conducted according to the principles expressed in the Declaration of Helsinki. All subjects read and signed the informed consent.

## Participants and Selection Criteria

The patients with facial acne were in the age range of 18–35 years. The severity of acne was classified as mild (grade I), moderate (grade II and III), and severe (grade IV), according to the Pillsbury grading system (Supplementary Material 1). Those patients with grade IV acne were enrolled in this clinical trial. Peripheral blood was collected before treatment.

### Exclusion Criteria Included

1) pregnancy and lactation, 2) a history of photoallergy, 3) active facial herpes simplex, 4) planning to have children, 5) consumed antibiotics, hormonal drugs, ISO, or photoallergic drugs within the last 1 year, 6) diabetes mellitus, organ defects of the heart, lung, liver and kidney, and 7) neurological or psychiatric disorders.

An oral ISO dose of 0.4 mg/kg/d was given for each patient. After 8-weeks of oral ISO treatment, nine acne vulgaris patients that presented different reactions to oral ISO were selected and divided into groups. Group A included those patients effectively treated by ISO, group B included those patients ineffectively treated by ISO, group C included those patients with ISO-induced acne flare-ups, and three healthy subjects were included as the control group, D. There were three patients in each group, and three healthy subjects in the control group. Peripheral blood was collected at the time of the 8-week oral ISO treatment.

This study was conducted according to the Declaration of Helsinki Principles. The patients were from the Dermatologic Clinic of the Third Affiliated Hospital of Sun-Yat sen University and healthy controls were volunteers from Sun-Yat sen Medical College. Written informed consents were obtained from all human subjects in this study. The study was approved by The Ethics Committee of the Third Affiliated Hospital of Sun-Yat sen University ([2018]02–194-01).

## Assessment of Oral ISO Therapeutic Effect

The number of inflammatory acne lesions, non-inflammatory acne lesions, and total acne lesions comprised of comedones, inflammatory papules, pustules, nodules, and cysts were evaluated by the investigators at day 0, day 14, day 28, day 42, and day 56.

The severity of acne vulgaris was graded depending on the Pillsbury grading system (Supplementary 1). Acne flare-up was defined as a lesion count that increased >30% compared to baseline (day 0, pre-treatment). Treatment was defined as effective when the lesion count decreased >30% compared to the baseline. Treatment was defined as ineffective when the lesion count increased <10% compared to the baseline.

## VISIA Capture and Analysis

Facial images were taken and analyzed at V0 (day 0), V1 (day 14), V12 (day 28) and V3 (day 56) via VISIA-CR skin tester (CANFIELD, Scientific Inc. USA)

## RNA Sequencing

PBMCs were isolated from peripheral blood and total RNA was extracted by using the PAXgene blood RNA kit (BD, USA). After the construction of cDNA libraries using the KAPA Stranded RNA-Seq Library Prep Kit

(Illumina, USA), RNA sequencing was performed on an Illumina HiSeq 4000 (Illumina, USA).

## Gene Ontology Analysis

Functional annotations of changing genes were carried out using Gene Ontology (GO). The Gene Ontology project provides a controlled vocabulary to describe gene and gene product attributes in any organism (http://www.geneontology.org). The ontology covers three domains: biological process, cellular component and molecular function. Fisher's exact test was used to find if there was more overlap between the list of different expressed genes and the GO annotation than that would be expected by chance. The enrichment analysis was processed with customized programs (python/R/shell, etc.) by KangChen Bio-tech, Shanghai, China.

## KEGG Analysis

Analysis of the molecular pathways of the genes was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/genes.html). The KEGG is the database of the genome and stores gene information related to metabolism, membrane transport, signal transduction, and the cell growth cycle. The enrichment of KEGG pathways was analyzed to find the relation of differentially expressed genes of acne patients with different reactions to ISO. The enrichment analysis was processed with customized programs (python/R/shell etc.) by KangChen Bio-tech, Shanghai, China.

## Quantitative Real-Time PCR

We observed an obvious change in seven genes identified from the differentially expressed gene profiles of ISO-induced acne flare-up patients and ISO-ineffective patients, which were then selected and quantified via qRT-PCR. The cDNA was generated from 2 μg of total RNA as described above, using the Gene Amp PCR System 9700 (Applied Biosystems. To run the PCR, 2X PCR master mix (Arraystar) and specific primers (designed by Primer 5.0) were used on a ViiA 7 Real-time PCR System (Applied Biosystems). Normalization was carried out using β-actin.

## Statistical Analysis

Data were analyzed using PASW Statistics (SPSS), version 18. Friedman test was used to analyze changes in the lesion counts. Statistical significance was attained when $P < 0.05$ and Q value <0.2 and R software Ballgown (v2.8.4) was used to sequence the differentially expressed genes. The level of gene expression was described as FPKM (fragments per kilobase of gene/script model per million mapped fragments), and genes with a mean FPKM greater than 0.5 in each group were taken for analysis. A $\log_2$ ratio of 0.585 corresponds to a 1.5-fold change ($|\log_2 fc| \geq 0.585$). Fisher's exact test with $p<0.05$ and FDR<0.05 was used as the cut-off standard in the GO and KEGG pathway enrichment analysis.

# Results

## Clinical Characteristics

In this study, 113 acne vulgaris patients were included, of which 9 acne patients (3 male and 6 female, aged 24±4.9, Pillsbury IV grade) were selected after 8-week oral ISO treatment. There were three patients (1 male, 2 females) in each group. There was no statistical difference in the subjects' age and gender between the oral ISO treatment group and the healthy control group. Additionally, there was no statistical difference in the acne severity scores, disease course, and oral ISO dosage between the three acne patient groups (Table 1).

## Changes in Gene Expression Post-Oral ISO Treatment

There were 39 changed genes, of which 31 were upregulated and 8 were downregulated, found when comparing

**Table 1** Study Groups and Clinical Information

| Group | Description | Gender | | Age | ISO DOS (Mg/Kg/) | Acne Severity Cores (Before Treatment) | Acne Duration (Year) |
|---|---|---|---|---|---|---|---|
| A | Effective treated by ISO | 2F | 1M | 23.7±6.1 | 0.4 | 162.13±33.7 | 4±3.8 |
| B | In-effective treated by ISO | 2F | 1M | 22.0±6.7 | 0.4 | 168.83±24.6 | 5±2.2 |
| C | ISO induced acne flare-up | 2F | 1M | 22.2±5.3 | 0.4 | 155.25±28.3 | 5±4.2 |
| D | Healthy control | 2F | 1M | 22.6±4.9 | / | / | / |

**Abbreviations:** F, female; M, male; ISO, isotretinoin.

post- and pre-ISO treatment in ISO-effective patients (Figure 1A). There were 345 changed genes, of which 292 were upregulated and 53 were downregulated, identified when comparing post- and pre-ISO treatment in ISO-ineffective patients (Figure 1B). There were 35 changed genes, of which 22 were upregulated and 13 were downregulated, when comparing post- and pre-ISO treatment in ISO-induced acne flare-up patients (Figure 1C).

## Characterization and Molecular Functional Analysis of Differentially Expressed Genes Identified by Comparing Post- and Pre-ISO Treatment

The circulating RNAs of patients in the ISO-effective group were compared and analyzed by GO, which showed that the 31 upregulated genes were mainly enriched in DNA-templated transcription, DNA repair, cellular response to DNA damage stimulus posttranscriptional gene, regulation of DNA-templated transcription, and regulation of transcription from the RNA polymerase II promoter, and the Wnt signaling pathway (Figure 2). KEGG analysis showed that the eight downregulated genes were enriched in negative regulation of the apoptotic process.

In the ISO-ineffective group, 292 upregulated genes were found to be primarily enriched in ribosome-related biological processes, and 53 upregulated genes were found to be involved in the cellular response to interferon-gamma. Several biological processes and pathways presented simultaneously positive and negative regulatory changes, and these included lymphocyte differentiation, protein ubiquitination, immune response, NF-κ B signal correlation, and the apoptosis signaling pathway.

In the ISO-induced acne flare-up group, we found 22 genes that were upregulated. Obvious changes were observed in the genes SMIM3, LY96, CD52, S100A12, and TICAM2, of which LY96, S100A12, and TICAM2 are involved in inflammation (Table 2). GO significant enrichment analysis showed significant enrichment in the toll-like receptor (TLR) signaling pathway, NF-κB signaling pathway, inflammatory response, and innate immune response, while the 13 downregulated genes were enriched in cell-matrix adhesion and so forth.

## ISO-Induced Acne Flare-Up Patients Compared with Healthy Control Subjects

Comparison of the gene profiles in the ISO-induced acne flare-up patients and healthy control subjects revealed expression changes in 57 genes. KEGG analysis reveals that 34 upregulated genes were enriched in the TNF signaling pathway and positive regulation of T cell differentiation, while GO analysis reveals that these genes were mainly involved in protease binding, transcription factor binding, and receptor binding in molecular functions, Golgi cellular component, positive regulation of regulatory T cell differentiation, positive regulation of cell adhesion, cellular defense response, cell surface receptor signaling pathway and cell-cell signaling in biological process (Figure 3A). Meanwhile, the 23 downregulated genes were found to be involved in antigen processing and presentation, cytokine production, and the TGF-beta receptor signaling pathway (Figure 3B).

## ISO-Ineffective Patients Compared with ISO-Induced Acne Flare-Up Patients

Comparison of the gene profiles of the ISO-induced-acne flare-up patients and the ISO-ineffective patients revealed the presence of 1778 upregulated genes, which were enriched in T cell differentiation, the Th17 cell differentiation pathway, the Th1 and Th2 cell differentiation pathway, T cell activation, the T cell receptor signaling pathway, and TOC signaling (Figure 4A). Genes involved in T cell differentiation and activation include the protein phosphatase family (PPP3CB, PPP3CC, PPP3R1), the interleukin family (IL7R, IL247, IL23A), the mitogen-activated protein kinase family (MAPK1, MAPK8, MAPK9), and CD247, CD3D, and CD3E. We also found that 57 downregulated genes were enriched in defense response to microbes (fungus, G± bacterium, protozoan, and virus), immune response (innate immune response in mucosa and antimicrobial humoral response), and neutrophil degranulation (Figure 4B). The Genes involved in the defense response to microbes included defensin coding gene DEFA1, DEFA3, MPO3, ELANE, and CTSG.

## Quantitative RT-PCR

Seven genes selected from differently expressed gene profiles of the ISO-induced acne flare-up group were quantified by qRT-PCR, which revealed that *GATA2, C4BPA,* and *CCR5* were up-regulated at 2.73 folds (P=0.024512), 35.87 folds (P=0.038073), and 2.48 folds (P=0.004681), respectively. Whereas *DEFA3, ELANE, MMP9*, and *RPS4Y1* were down-regulated to 0.18 fold (P=0.041934), 0.14 fold (P=0.030767), 0.41 fold (P=0.013383), and

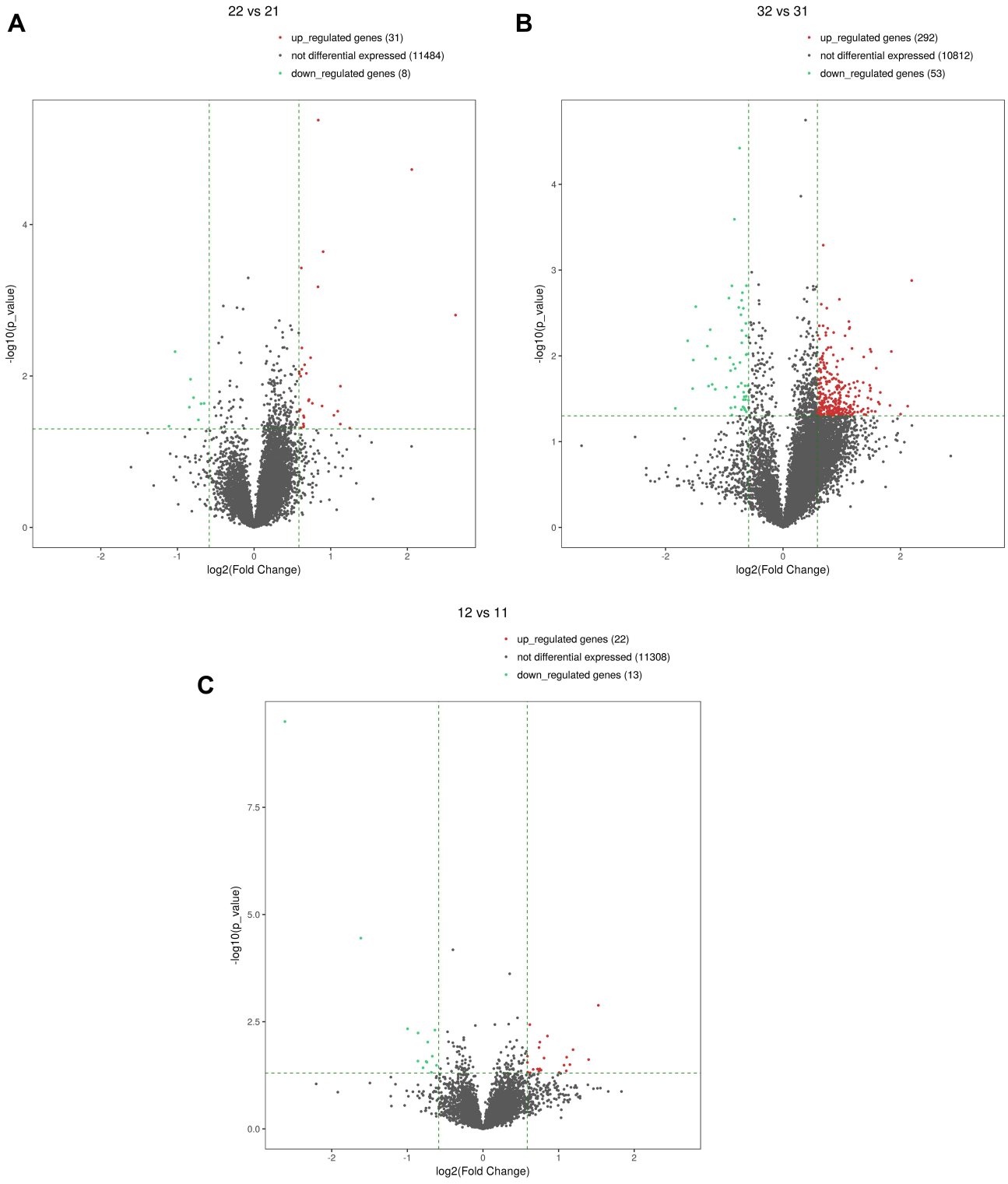


**Figure 1** Differentially expressed genes identified by comparing pre- and post-oral ISO treatment. After 8-week oral ISO treatment, 9 acne vulgaris patients presented different reactions to oral ISO, with 3 healthy subjects serving as the control. Peripheral blood was collected pre-treatment and after 8-week oral ISO. RNA was extracted from the peripheral blood and sequenced on an Illumina HiSeq 4000 (Illumina, USA). In the unsupervised cluster of changed genes, FC≥1.5 and p≤0.05 was considered as statistically significant. (**A**) Differentially expressed genes in ISO-effective patients. Of the 39 changed genes found in ISO-effective patients comparing post- and pre-ISO treatment, 31 were upregulated and 8 were downregulated. (**B**) Differentially expressed genes in ISO in-effective patients. Of the 345 changed genes identified in ISO-ineffective patients comparing post- and pre-ISO treatment, 292 were upregulated and 53 were downregulated. (**C**) Differentially expressed genes in ISO-induced acne flare-up patients. Of the 35 changed genes in ISO-induced acne flare-up patients comparing post- and pre-ISO treatment, 22 were upregulated and 13 were downregulated.

0.00018 fold (P=0.000986), respectively, when compared with the ISO-ineffective group (Table 3) ($p<0.05$).

## Discussion

The role of influencing the expression of inflammatory factors and the biological process of inflammation by oral ISO therapy has been identified by previous research. Becker et al found that ISO could activate IL-10 signaling in regulatory T-cells (Tregs) and naive T-cells, and that, in mice, it reduced effector T-cell proliferation alone and in co-culture with Tregs.[14] Borovaya et al found that, while elevated in untreated acne vulgaris, cutaneous expression of cathelicidin and koebnerisin returned to normal levels after 6 months of ISO therapy.[15] Furthermore, Perkins et al suggested that 13-cis-retinoic acid might exacerbate acne in the initial stage via neutrophil-mediated inflammatory processes, which are driven by the decrease of lipid production and the death of P. acnes caused by the drugs.[16] In this study, we confirmed the findings of previous studies that revealed an upregulated inflammatory response and immune response. Changes in the expression of genes regulating the inflammatory response could be detected in all acne patients receiving ISO treatment, regardless of the efficacy of therapy. Further comparison of patients who responded variously to ISO therapy

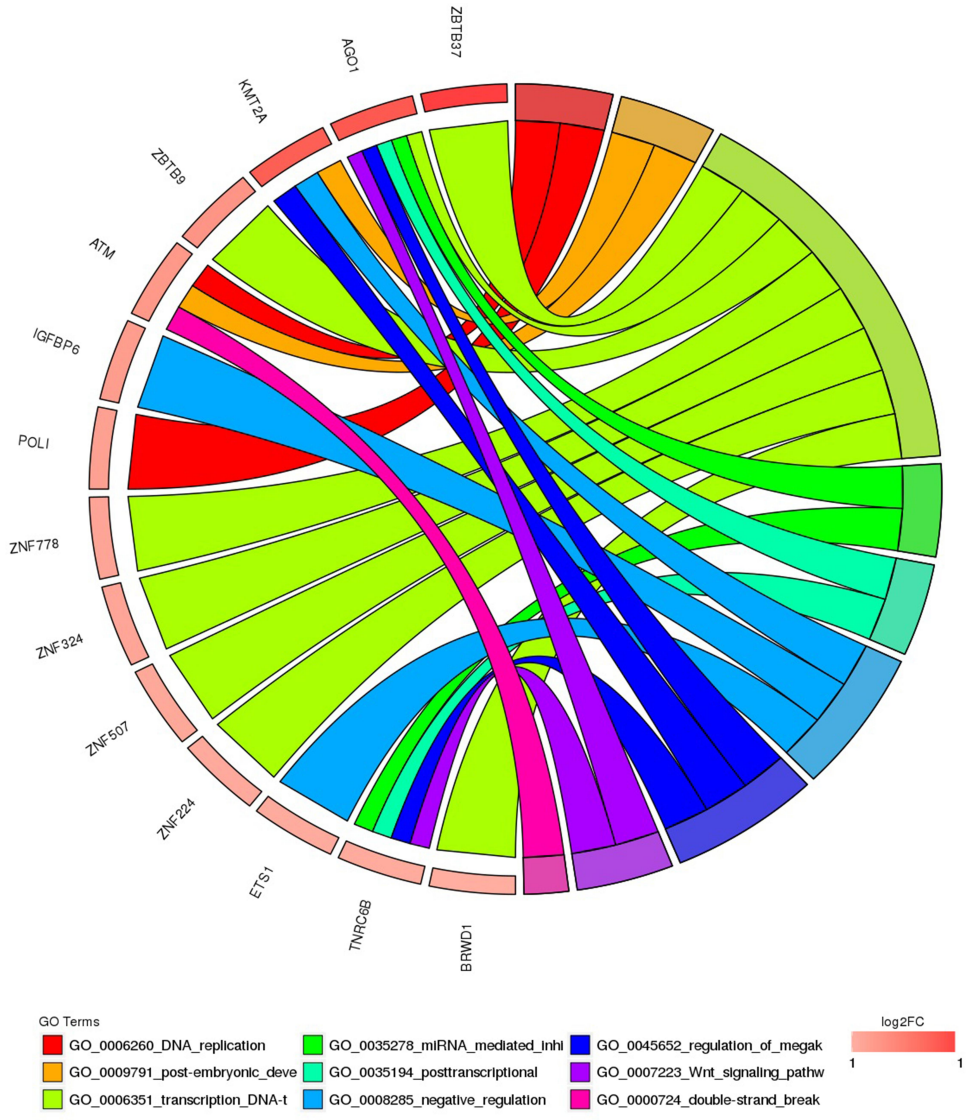


**Figure 2** Molecular functional analysis circulating RNA of patients presented as effective to ISO therapy. The peripheral blood of patients presented as effective to 8-week oral ISO therapy was collected before and after treatment. Circulating RNAs were compared and analyzed by GO which showed that changed genes were mainly involved in DNA-templated transcription, DNA repair, the cellular response to DNA damage stimulus posttranscriptional gene silencing by RNA, regulation of DNA-templated transcription and regulation of transcription from the RNA polymerase II promoter and Wnt signaling pathway in biological Process.

**Table 2** Obviously Changed Genes Identified by Comparing Pre- and Post-Isotretinoin Treatment in Acne Flare-Up Patients

| Gene Code | Gene | Fold Change | Gene Function Description |
|---|---|---|---|
| ENSG00000256235.1_2 | *SMIM3* | 2.88 | Small integral membrane protein 3 |
| ENSG00000154589.6_2 | *LY96* | 2.63 | Lymphocyte antigen 96 |
| ENSG00000169442.8_2 | *CD52* | 2.28 | CD52 molecule |
| ENSG00000163221.8_2 | *S100A12* | 2.22 | S100 calcium binding protein A12 |
| ENSG00000243414.5_3 | *TICAM2* | 2.15 | Toll-like receptor adaptor molecule 2 |
| ENSG00000118707.9_3 | *TGIF2* | 0.33 | TGFB-induced factor homeobox 2 |
| ENSG00000179348.11_3 | *GATA2* | 0.50 | GATA binding protein 2 |
| ENSG00000101162.3_2 | *TUBB1* | 0.55 | Tubulin, beta class 1 |
| ENSG00000259207.7_3 | *ITGB3* | 0.58 | Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) |
| ENSG00000173011.11_3 | *TADA2B* | 0.59 | Transcriptional adaptor 2B |

**Notes:** DEGs based on cut-offs as ≥1.5 FC, p≤0.05.
**Abbreviations:** DEG, differentially expressed gene; FC, fold change; GRCh37, reference genome.

enabled us to identify changes in genes that regulated the activity and function of Th17 cells and Toll-like receptor (TLR).

Th17 cells induce tissue inflammation through the production of IL-17, IL-6, and TNF-α, and are involved in acne pathogenesis.[17] Agak et al identified the antimicrobial activity of Th17 cells induced by P. acnes strains was associated with both healthy and acne skin and revealed that P. acnes could induce the immune response of Th17 and Th1 cells to produce IL-17 and IFN-γ.[18] Besides, supernatants from P. acnes-stimulated peripheral blood mononuclear cells (PBMCs) could promote the differentiation of naïve CD4+CD45RA T cells into Th17 cells. We compared ISO-induced acne flare-up and ISO-ineffective patients and found that 1778 genes were upregulated, and these were enriched in T cell differentiation, the Th17 cell differentiation pathway, the Th1 and Th2 cell differentiation pathway, T cell activation, and the T cell receptor signaling pathway. This finding suggested that the level of T cell gene expression induced by ISO affected the inflammatory responses to microorganism in skin lesions, thus resulting in the different therapeutic responses.

Toll-like receptor (TLR) is a pattern recognition receptor (PRR) and plays a key role in the innate immune system by recognizing the pathogen-associated molecular pattern (PAMP) on the surface of the antigen. Recent findings have supported that TLRs are associated with acne pathogenesis, including TLR-2 and TLR-4. Ozlu et al found that the level of TLR-2 immunohistochemical staining in various types of lesions (comedones, papules, pustules, and nodules) in the inflammation area, dermis, and skin appendages was higher than in the control group.[19] Additionally, Thiboutot et al found higher TLR-2 and lower TLR-4 levels in monocytes of acne patients compared to healthy subjects.[20] Yamamoto et al revealed that the TLR-4 mediated MyD88-independent pathway could also activate the NF-κB signaling pathway to induce the production of proinflammatory cytokines, as well as activate the promoter for the gene encoding IFN-β. Furthermore, TLR-2 can particularly recognize Gram-positive bacteria (P. acnes) and yeasts.[21] Jugeau et al found that TLR-4 can recognize Gram-positive bacteria through lipoteichoic acid (LTA) as well.[22] Through gene expression analysis, we found that two upregulated genes were enriched in the TLR signaling pathway, NF-κB signaling pathway, inflammatory response, and innate immune response in ISO-induced acne flare-up patients. This result suggested that, besides TLR, the inflammatory response and innate immune response also take part in the mechanisms of ISO-induced acne flare-up.

Our study also found that, in addition to regulating the inflammation reaction, patients who are undergoing ISO treatment showed activation of DNA damage repairing genes. In patients who respond well to ISO, we found 31 upregulated genes were enriched in DNA-templated transcription, DNA repair, the cellular response to DNA damage stimulus posttranscriptional gene silencing by RNA, regulation of DNA-templated transcription, and regulation of transcription from RNA polymerase II promoter and the Wnt signaling pathway. This suggested that activation of the DNA repair process might be involved in the ISO therapeutic mechanisms, especially in patients with ideal therapeutic effects.

It is thought that skin microbe–host interactions contributed to the mechanisms of facial diseases.[23] The host defense response can inhibit and resist P. acne. The downregulation of defense response-related pathways could

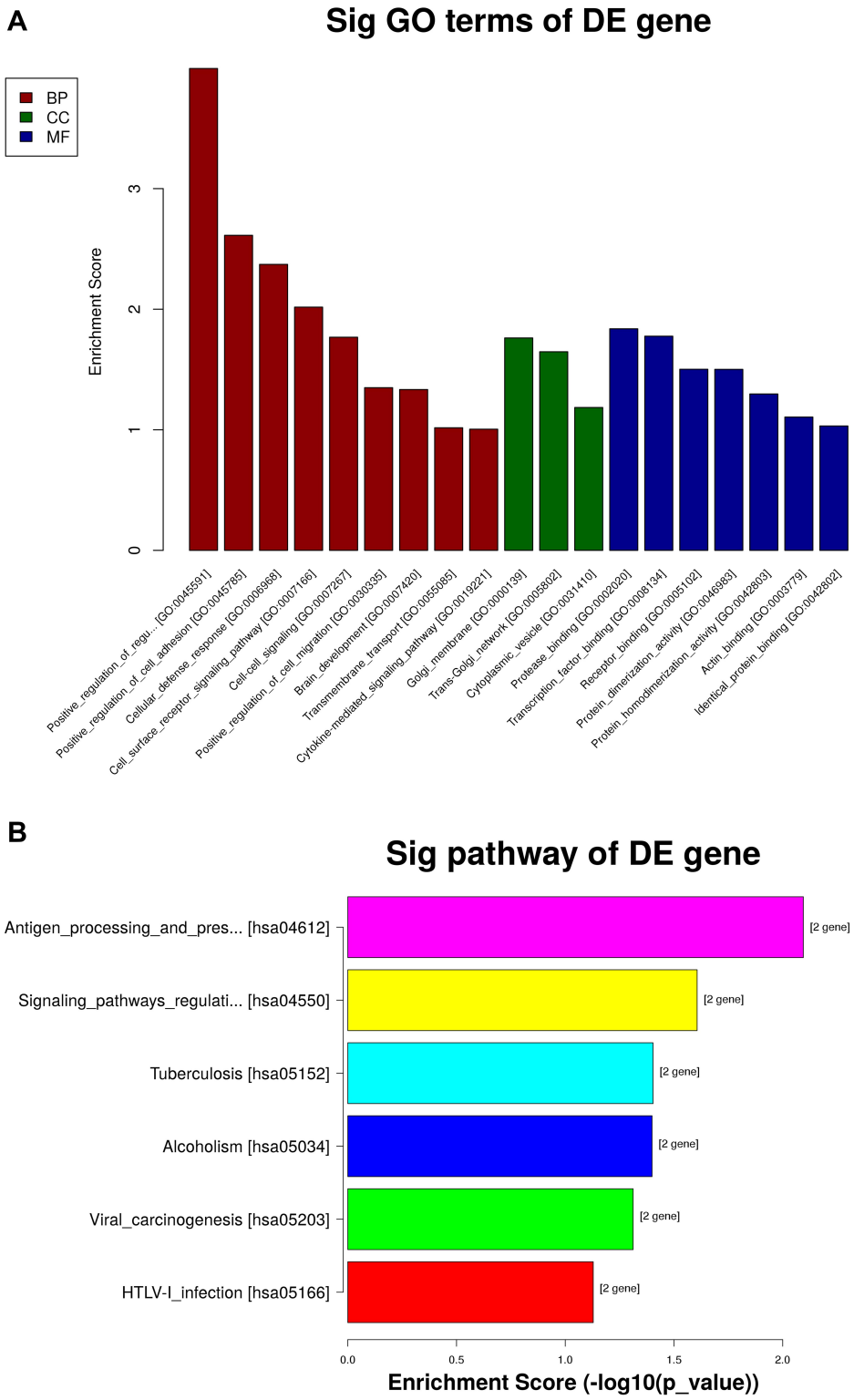


**Figure 3** Characterization and molecular functional analysis of differentially expressed genes comparing ISO induced-acne flare-up patients with healthy controls. Comparison of the gene profiles of ISO-induced acne flare-up patients and healthy control subjects revealed expression changes in 57 genes. Molecular pathways analysis of the genes was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/genes.html). Functional annotations for the genes were carried out using Gene Ontology (GO). The Gene Ontology project provides a controlled vocabulary to describe gene and gene product attributes in any organism (http://www.geneontology.org). (**A**) GO significant enrichment analysis revealed that the 34 upregulated genes were mainly involved in protease binding, transcription factor binding, and receptor binding in molecular function, Golgi cellular component; positive regulation of regulatory T cell differentiation, positive regulation of cell adhesion, cellular defense response, cell surface receptor signaling pathway and cell-cell signaling in biological process. (**B**) The 23 downregulated genes were involved in antigen processing and presentation, cytokine production, and the TGF-beta receptor signaling pathway.

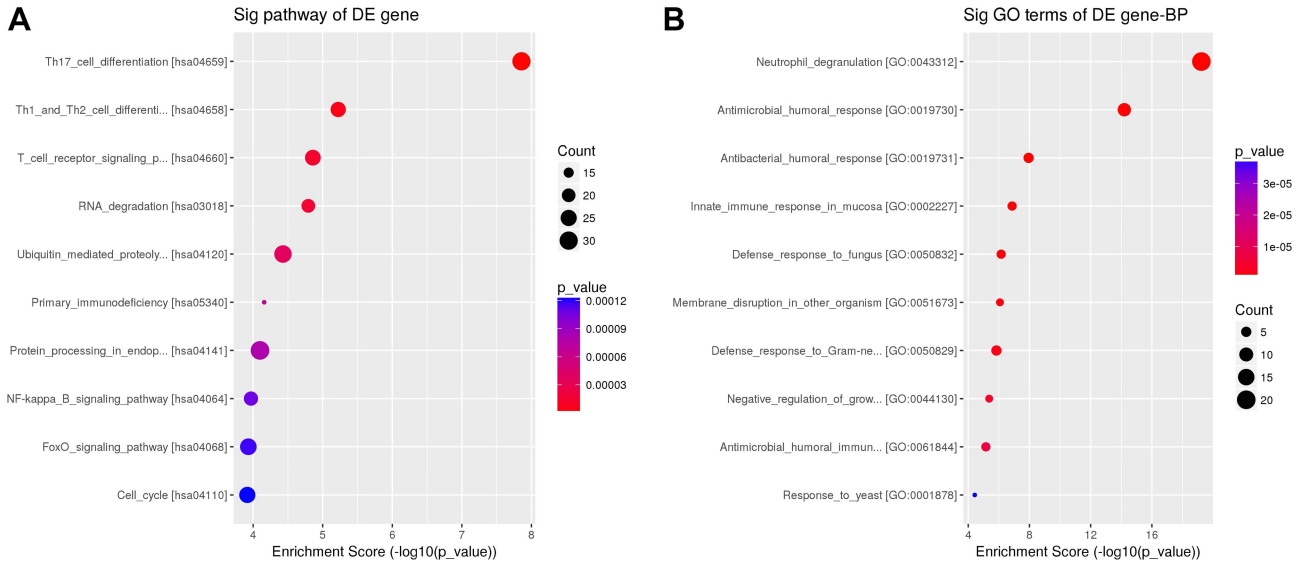


**Figure 4** Biological and functional analysis of changed genes found when comparing the ISO-induced acne flare-up group and the ISO-ineffective group. Peripheral blood RNA from ISO-induced acne flare-up patients and ISO-ineffective patients was extracted, sequenced, and then analyzed via KEGG and GO. (**A**) KEGG pathway analysis showed differentially expressed genes were enriched in T cell differentiation, the Th17 cell differentiation pathway, the Th1 and Th2 cell differentiation pathway, T cell activation, the T cell receptor signaling pathway, and TOC signaling. (**B**) GO significant enrichment analysis revealed that downregulated genes were mainly involved in defense response to microbes (fungus, G± bacterium, protozoan, and virus), immune response (innate immune response in mucosa and antimicrobial humoral response), and neutrophil degranulation.

cause microorganism colon increases, especially P. acnes in acne patients. We found that genes enriched in the defense response to microbes (fungus, G± bacterium, protozoan, and virus), immune response (innate immune response in mucosa and antimicrobial humoral response), membrane disruption in other organisms, and killing of cells of other organisms were downregulated in acne flare-up patients. This might indicate that ISO-induced suppression of immune defense response genes might aggravate acne, as microbial colonization was abnormally increased. However, the microbiological mechanisms still need further investigation of the skin microflora.

By comparing patients with extreme reactions to ISO therapy, acne flare-up and in-effect (no response to ISO treatment), we found obvious changes in the *GATA2, C4BPA, CCR5, DEFA3, ELANE, MMP9*, and *RPS4Y1* genes. DEFA3 regulated defensin, alpha 3, and neutrophil-specific factor. *ELANE* regulated elastase and neutrophils. *MMP9* regulated matrix metalloproteinase-9 expression. *RPS4Y1* regulated ribosomal protein S4 and Y-linked 1 was downregulated, while *GATA2* was involved in the transcriptional process. *C4BPA* was involved in the negative regulation of the complement system, while *CCR5* involved in the chemotaxis signaling pathway and the cytokine response process were upregulated. This finding indicated that genes regulating the inflammatory response and microorganism defense response played important roles in affecting ISO therapeutic reactions. Overreaction of inflammatory responses includes activating T cell differentiation, the T cell receptor signaling

**Table 3** qPCR of Change Genes of ISO-Induced Acne Flare-Up Patients vs ISO-Ineffective Patients

| **Genes** | **GATA2** | **C4BPA** | **CCR5** | **DEFA3** | **ELANE** | **MMP9** | **RPS4Y1** |
|---|---|---|---|---|---|---|---|
| **Gene Description** | **GATA Binding Protein 2** | **Complement Component 4 Binding Protein, Alpha** | **Chemokine (C-C Motif) Receptor 5** | **Defensin, Alpha 3, Neutrophil-Specific** | **Elastase, Neutrophil Expressed** | **Matrix Metalloproteinase-9** | **Ribosomal Protein S4, Y-Linked 1** |
| Up/Down | Up | Up | Up | Down | Down | Down | Down |
| Change Fold | 2.73 | 35.87 | 2.48 | 0.18 | 0.14 | 0.41 | 0.00018 |
| P value | 0.024512 | 0.038073 | 0.004681 | 0.041934 | 0.030767 | 0.013383 | 0.000986 |

pathway, and the NF-κB signaling pathway, along with inadequate defense response to microorganisms, including immune response (innate immune response of mucous membrane and humoral immune response) might induce acne flare-up.

The analysis of RNAs in circulating blood of ISO-ineffective acne patients revealed that several biological processes and pathways simultaneously presented positive and negative regulation, including lymphocyte differentiation, protein ubiquitination, immune response, NF-κB signal correlation, the apoptosis signal pathway, and so on. This might suggest that self-offsetting biological effects might contribute to the mechanisms of ISO therapy insensitivity, which still needs further investigation in the way of molecular biology and immunological study.

There are several limitations to the study. The study population consists of university dermatology clinic patients in South China, and may not be representative of patients in the general population. Although there is little clinical relevance to help us in our daily care of isotretinoin patients, it expands our knowledge of this important drug. Larger sample size research carried out in future would help to further understand the role of gene expression in affecting oral isotretinoin therapeutic efficacy in acne vulgaris patients.

In conclusion, we found that isotretinoin could temporarily alter gene expression in acne patients, and the gene profiles among oral ISO-induced acne flare-up, effective, and ineffective patients were distinct. ISO therapeutic mechanisms were not only involved in regulating the inflammatory reaction but also in the process of DNA repair. Genes that regulated the inflammatory and defense response to microorganisms played a role in ISO treatment effects. *GATA2, C4BPA, CCR5, DEFA3, ELANE, MMP9,* and *RPS4Y1* might be the susceptible genes implicated in ISO-induced acne aggravation. These findings revealed the genetic roles in the ISO treatment process and provided a basis for exploring precise personalized treatment plans for acne patients.

## Abbreviations

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TLR, toll-like receptor; FC, fold change; FDR, false discovery rate.

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## Disclosure

The authors report no conflicts of interest for this work.

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