ISSN 2304-3415, Russian Open Medical Journal
2024. Volume 13. Issue 1 (March). Article CID e0110
DOI: 10.15275/rusomj.2024.0110


Original article

# Expression dynamics of cytokine genes is related to the apremilast treatment effectiveness in patients with severe psoriasis

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*Received 19 August 2022, Revised 12 September 2023, Accepted 1 December 2023*



**Abstract:** *Background* — Psoriasis is an immune-mediated genetic skin disease with a deregulated immune response governed by a proinflammatory cytokine network. Apremilast has demonstrated high safety and tolerability both in clinical trials and in clinical practice. The effectiveness of the apremilast use in clinical practice may differ from major clinical trials. Our study assessed changes in the levels of immune gene expression in patients suffering from severe psoriasis in the course of apremilast treatment in order to investigate the predictors of its effectiveness.

*Methods* — We assessed the expression levels of *IFNγ, IRF3, GLIS1, HR, STAT1, STAT3, VEGFA, ICAM1, TNF, IL1α, IL1β, IL4, IL6, IL10, IL11, IL12B, IL17A, IL17F, IL18, IL20, IL21, IL22, IL23A, IL25, IL31, IL33* genes in both lesional and nonlesional skin before the treatment, as well the expression at lesional skin after the treatment. RNA expression was assessed in skin biopsy samples by RT-PCR using TaqMan probes with StepOne5 equipment and normalized with endogenous control. The study included 16 patients diagnosed with a moderate-to-severe or severe psoriasis using clinical examination by a dermatologist. The clinical outcome after 26 weeks of apremilast treatment was assessed with delta PASI, resulting in a patient group with high effectiveness of treatment (delta PASI>75%) and a group including all other patients.

*Results* — We confirmed elevated levels of expression in *STAT1, IFNγ, IL1β, IL12B, IL17A, IL17F, IL20, IL21, IL22*, and *IL23A* genes in lesional vs. nonlesional psoriatic skin samples, while *GLIS1* gene expression was reduced. The expression levels of cytokine genes after apremilast treatment decreased considerably in cytokines *IFNγ, IL1β, IL20, IL21*, and *IL22*; and to a lesser extent in *STAT1, IL6, IL17F, IL22* and *IL31*. In the group of those who effectively responded to treatment with apremilast, a five-to-eleven-fold reduction in the expression level of the *IL1B, IL6*, and *IL17F* genes was observed, as compared with other patients.

*Conclusion* — The increased expression of cytokine genes in lesional vs. nonlesional skin was reduced after apremilast treatment of psoriasis. We established that fold changes in the expression of the *IL1β, IL6* and *IL17F* genes during treatment with apremilast were different in groups of patients with different therapy outcomes. Hence, we propose that they are the predictors of the effectiveness of apremilast treatment for severe psoriasis.

**Keywords:** apremilast, psoriasis, gene expression, therapy outcome, cytokine.

*Cite as* Verbenko DA, Karamova AE, Artamonova OG, Kozlova IV, Deryabin DG, Solomka VS, Kubanov AA. Expression dynamics of cytokine genes is related to the apremilast treatment effectiveness in patients with severe psoriasis. *Russian Open Medical Journal* 2024; 13: e0110.

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## Introduction

Psoriasis is an immune-mediated genetic skin disease that affects 2-3% of the population worldwide [1-3]. This condition is characterized by abnormal keratinocyte hyperproliferation and differentiation related to deregulated response of the immune system governed by a proinflammatory cytokine network [4]. It is generally believed that inflammation in psoriasis lesions is caused by the Th1 pathway with considerable influence of Th17 and Th23 cells [5-7]. Knowledge of molecular pathogenesis would allow developing target approaches to psoriasis treatment.

Considerable success in psoriasis treatment was achieved with the application of immunosuppressant drugs (biologics), particularly selective inhibitors of *TNF, IL17, IL12,* and *IL12/23[2]*. Selective inhibitors of phosphodiesterase-4 activity, such as apremilast, rolipram, crisaborole, roflumilast, etc., were developed for anti-inflammatory targeted therapy, including treatment of severe psoriasis [8].

Apremilast is a phosphodiesterase-4 inhibitor approved by The United States Food and Drug Administration (FDA) for the treatment of moderate-to-severe plaque psoriasis [9-10]. PDE4 inhibition increases the intracellular concentration of cyclic adenosine monophosphate (cAMP), which results in decrease of the proinflammatory response via reducing Th1, Th17, and interferon pathways [11]. This, in turn, lowers the production of proinflammatory cytokines, such as *TNF-α, IL-2, IL-8, IL-12, IL-23,* and *IFN-γ*, simultaneously increasing the production of IL-6 and IL-10 that suppress inflammation [12].

In addition, its use provides some improvement in the condition of patients with psoriatic arthritis [13], has a beneficial effect on biologically unresponsive patients [14] and on the comorbidities of psoriasis [15], along with improving the treatment of psoriasis with methotrexate [16]. Safety profile of apremilast is more promising than treatment with biologics, although a higher incidence of poor response and low adherence to treatment were described [17-20], which determines the relevance of searching for predictors of apremilast effectiveness. Whereas the search for such predictors was made by pharmacogenetics [17, 21] and proteomic [16] research, studies of gene expression patterns associated with the clinical outcomes of apremilast treatment were not conducted at all or were rather limited. The goal of our study was to assess changes in the expression level of cytokine genes in the skin of patients with moderate-to-severe or severe psoriasis with different outcomes of apremilast treatment

## Material and Methods

### *Psoriatic patient cohort*

Clinical characteristics of the patients such as phenotype classification, exclusion and inclusion criteria, clinical assessment of psoriasis severity including Psoriasis Area and Severity Index (PASI) score, and targeted therapy for psoriasis using apremilast were assessed as previously described [16, 17].

The flow chart of the study design is shown in *Figure* 1. Clinical assessment of PDE-4 inhibitor efficacy was carried out using ΔPASI indices at week 26 after the onset of a targeted therapy. In order to search for predictors of apremilast treatment effectiveness, two study groups were formed: patients with a positive response to the drug (ΔPASI≥75%, 8 patients) and the rest (ΔPASI≤50%, 8 patients).

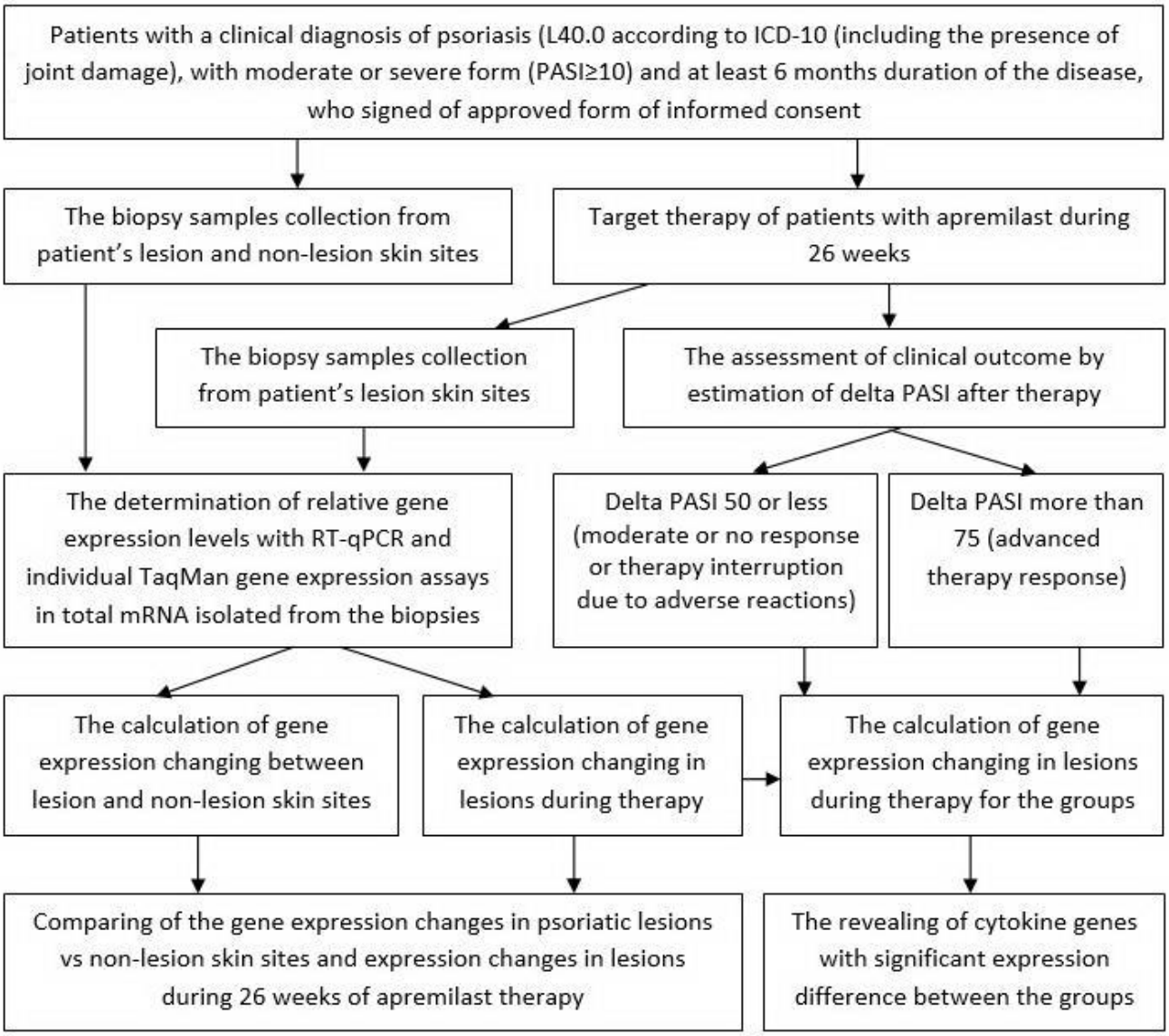



**Figure 1. Flow chart for the study.**

ICD-10, International Statistical Classification of Diseases and Related Health Problems according to World Health Organization; PASI, Psoriasis Area and Severity Index; mRNA, messenger ribonucleic acid; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

**Table 1. The fold change of gene expression in psoriasis lesions (after vs. before treatment) in psoriatic patient groups with different apremilast treatment outcomes: median (range of values).**

| *Gene* | *Beneficial outcome of apremilast treatment (delta PASI>75)* | *Other outcomes of apremilast treatment* | *ROC analysis AUC and P-value* |
|---|---|---|---|
| *IFNY* | 0.23 (0.02-3.6) | 0.38 (0.13-2.1) | 0.563 (P=0.703) |
| *IRF3* | 0.8 (0.15-4.96) | 1.12 (0.64-2.55) | 0.672 (P=0.243) |
| *GLIS1* | 1.46 (0.005-176) | 1.74 (0.36-152.7) | 0.578 (P=0.614) |
| *HR* | 0.77 (0.286-5.2) | 0.93 (0.63-10.2) | 0.625 (P=0.435) |
| *STAT1* | 0.58 (0.1-3.1) | 0.68 (0.24-3.27) | 0.547 (P=0.762) |
| *STAT3* | 0.67 (0.3-2.8) | 0.79 (0.4 -1.2) | 0.594 (P=0.561) |
| *VEGFA* | 1.2 (0.3-2.5) | 0.84 (0.45-1.65) | 0.578 (P=0.643) |
| *ICAM1* | 0.59 (0.1-2) | 1.26 (0.15-1.7) | 0.625 (P=0.425) |
| *TNF* | 1.1 (0.1-20.8) | 1.9 (0.46-3.6) | 0.641 (P=0.352) |
| *IL1A* | 0.4 (0.075-2.9) | 0.83 (0.1-2.4) | 0.625 (P=0.409) |
| *IL1B* | 0.12 (0.02-0.93) | 0.68 (0.14-1.4) | 0.857 (P=0.002) |
| *IL4* | 0.29 (0.036-2) | 1.60 (0.38-4.76) | 0.768 (P=0.065) |
| *IL6* | 0.12 (0.02 -1.6) | 1 (0.16-13.6) | 0.786 (P=0.028) |
| *IL10* | 0.36 (0.16-2.3) | 0.86 (0.2-1.6) | 0.688 (P=0.221) |
| *IL11* | 1.8 (0.09-16.2) | 0.68 (0.35-1.55) | 0.750 (P=0.086) |
| *IL12B* | 0.5 (0.03-11) | 0.73 (0.06-27) | 0.5 (P=1) |
| *IL17A* | 0.51 (0.001-6.4) | 0.57 (0.003-1.45) | 0.531 (P=0.852) |
| *IL17F* | 0.07 (0.005-1.2) | 0.78 (0.05-1.9) | 0.768 (P=0.037) |
| *IL18* | 1.46 (0.09-5) | 1.16 (0.6-3.1) | 0.578 (P=0.629) |
| *IL20* | 0.51 (0.003-3.5) | 0.34 (0.2-1.9) | 0.516 (P=0.923) |
| *IL21* | 0.15 (0.01-11.5) | 0.3 (0.04-2) | 0.578 (P=0.633) |
| *IL22* | 0.45 (0.0003-8.4) | 0.67 (0.05-1.6) | 0.531 (P=0.845) |
| *IL23A* | 0.76 (0.17-8.6) | 0.57 (0.27-2.8) | 0.531 (P=0.851) |
| *IL25* | 1.1 (0.16-37.9) | 1.4 (0.46-2019.8) | 0.609 (P=0.485) |
| *IL31* | 0.3 (0.001-342.5) | 0.7 (0.015-1.68) | 0.531 (P=0.863) |
| *IL33* | 1.2 (0.4-5.6) | 1.17 (0.4-4) | 0.547 (P=0.767) |

### *Sample collection and RNA isolation*

We collected 48 skin samples from 16 patients with moderate-to-severe or severe psoriasis using standard 5 mm punch biopsy from psoriatic lesions before and after 26 weeks of apremilast treatment, as well from visually clean (nonlesional) skin before treatment. The skin biopsy specimens were placed in 1 mL of Allprotect Tissue Reagent (QIAGEN, Germany) immediately after sampling and were treated according to the manufacturer protocol. Total mRNA extraction was performed with an Easy RNA kit (QIAGEN, Germany) from biopsy specimens treated with Allprotect Tissue Reagent. Quantity and quality of mRNA were assessed using NanoVue spectrophotometer (General Electric, France).

### *Assessment of gene expression level*

RNA expression was assessed in single-tube reactions using FAM labeled gene expression TaqMan assays with QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) and Quant Studio Design & Analysis Software v. 5.0 (Thermo Fisher Scientific, USA). The expression levels of *IFNγ, IRF3, GLIS1, HR, STAT1, STAT3, VEGFA, ICAM1, TNF, IL1A, IL1B, IL4, IL6, IL10, IL11, IL12B, IL17A, IL17F, IL18, IL20, IL21, IL22, IL23A, IL25, IL31,* and *IL33* genes were investigated with the following TaqMan Gene Expression Assays (Thermo Fisher Scientific, USA): *IL-1α* (Hs00174092_m1), *IL-1β* (Hs00174097_m1), *IL-4* (Hs00174122_m1), *IL-6* (Hs00985639_m1), *IL-10* (Hs00961622_ m1), *IL-11* (Hs01055413_g1), *IL-12* (Hs01011518_m1), *IL-17A* (Hs00177383_m1), *IL-17F* (Hs00369400_m1), *IL-18* (Hs01038788_m1), *IL-20* (Hs00218888_m1), *IL-21* (Hs00222327_m1), *IL-22* (Hs01574154_m1), *IL-23* (Hs00900828_g1), IL-25 (Hs03044841_m1), *IL-31* (Hs01098710_m1), IL-33 (Hs00369211_m1), *ICAM-1* (Hs00164932_m1), *VEGFA* (Hs00900055_m1), *IFN-γ* (Hs00989291_m1), *TNF-α* (Hs01113624_g1), *STAT1* (Hs01013996_m1), *STAT3* (Hs00374280_m1), *GLIS1* (Hs01672213_m1), *HR* (Hs00218222_m1), and *IRF3* (Hs00218222_m1). The *GAPDH* (cat. #4333764T, Thermo Fisher Scientific, USA) and *HPRT1* (cat. #4326321E) genes were used as endogenous control. The VIC-tagged HPRT1 gene expression assay was added to each reaction mixture, and the GAPDH gene expression assay was analyzed as a separate reaction mixture, along with the target genes for each mRNA sample in the same RT-qPCR run.

Raw data on mRNA levels were obtained using one-step RT-qPCR with reaction mixture that allowed simultaneous cDNA reverse transcription followed by TaqMan qPCR in one tube. The reaction mixture contained 5 µL of TaqPath™ 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific, USA), 1µL of target TaqMan gene expression assay, 1 µL of total mRNA solution and RNAse-free water up to 20µL volume. RT-qPCR conditions and fluorescent detection complied with the manufacturer protocol. RT-qPCR experiments were repeated at least twice.

### *Fold change of gene expression*

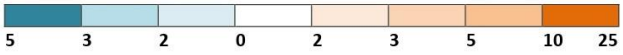
The relative values of PCR expression were obtained as delta cycle threshold (Ct) against the *GAPDH* endogenous control, which was chosen due to a reduced threshold cycle, as compared to *HPRT1*. Fold change values of gene expression (RQ) were calculated with Quant Studio Design & Analysis Software v.5.0 (Thermo Fisher Scientific, USA) according to the double-delta Ct method [22]. First of all, delta Ct for each examined gene was calculated by the formula: ΔCt = Ct (gene of interest) – Ct (housekeeping gene). Secondly, Ct values were calculated as follows: ΔΔCt = ΔCt (treated sample of lesional skin) – ΔCt (untreated sample of lesional skin) or ΔΔCt = ΔCt (sample of lesional skin) – ΔCt (sample of nonlesional skin). Fold change values of gene expression are calculated by the formula $R=2^{-\Delta\Delta Ct}$. The fold change values in gene expression based on Ct (double-delta cycle threshold) were calculated for each sample. The median values are presented in the tables (with the range from minimum to maximum value indicated in parentheses).

### *Statistical data processing*

Descriptive statistics were obtained with MedCalc® Statistical Software version 20.218 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023). The quantitative RQ data did not follow a normal distribution and therefore, gene expression values are presented as median in *Table* 1 and *Figure* 2. The statistical significance of differences in the expression levels of the studied genes between groups was determined using ROC analysis, in which the best sensitivity and specificity are observed when using expression levels as a classifier. Based on the fold change values of gene expression set as a classifier, the software calculates a cut-off threshold value (separating the groups with different outcomes of apremilast treatment of psoriasis), thereby achieving maximum values of the total sensitivity and specificity of the test. Statistically significant differences were assumed at $p<0.05$.

| Gene | In lesion vs visually clean skin | After vs before the therapy |
|---|---|---|
| *IFNY* | 4.46 (0.09-15.7) | 0.3 (0.02-3.6) |
| *IRF3* | 0.6 (0.1-1.8) | 0.9 (0.15-5) |
| *GLIS1* | 0.35 (0.006-2.7) | 1.6 (0.005-176) |
| *HR* | 1.1 (0.5-3.2) | 0.9 (0.3-10.2) |
| *STAT1* | 2.5 (0.6-5.4) | 0.6 (0.1-3.2) |
| *STAT3* | 1.6 (0.6-2.4) | 0.7 (0.3-2.7) |
| *VEGFA* | 0.86 (0.3-1.8) | 1 (0.3-2.5) |
| *ICAM1* | 0.98 (0.26-1.7) | 0.75 (0.1-2) |
| *TNF* | 0.96 (0.16-2.3) | 1.6 (0.1-20.8) |
| *IL1A* | 1.2 (0.4-4.1) | 0.7 (0.07-2.9) |
| *IL1B* | 5.8 (3-24) | 0.3 (0.02-13.7) |
| *IL4* | 0.9 (0.016-4.4) | 1.3 (0.04-6.2) |
| *IL6* | 1.5 (0.2-7.2) | 0.5 (0.02-13.6) |
| *IL10* | 1.5 (0.5-3.2) | 0.6 (0.16-2.3) |
| *IL11* | 0.6 (0.2-1.8) | 0.9 (0.09-16) |
| *IL12B* | 12 (0.4-185) | 0.9 (0.03-27) |
| *IL17A* | 26.7 (1.5-563.4) | 0.6 (0.001-6.4) |
| *IL17F* | 23 (9.2-65) | 0.5 (0.004-3.8) |
| *IL18* | 0.6 (0.5-10.5) | 1.2 (0.09-5) |
| *IL20* | 13 (0.3-255) | 0.4 (0.003-3.4) |
| *IL21* | 24.8 (1-207) | 0.2 (0.01-11.6) |
| *IL22* | 10 (4.4-233) | 0.5 (0.0003-8.4) |
| *IL23A* | 2.9 (0.4-8.9) | 0.6 (0.15-8.6) |
| *IL25* | 1.9 (0.3-6) | 1.4 (0.16-2019.8) |
| *IL31* | 0.9 (0.004-10.8) | 0.5 (0.001-342.6) |
| *IL33* | 1.9 (0.5-2.9) | 1.2 (0.4-5.6) |

**Gene expression fold change heatmap legend**




Blue color designated reducted, brown designates elevated value

**Figure 2. Gene expression change in psoriasis lesions (in lesion/visually clean skin) and in lesions in the course of apremilast treatment (after/before the therapy). The shadowed cells indicate twofold or greater changes in the expression (see heatmap legend).**

## Results

We studied gene expression of cytokines (*IFNγ*, *TNF*), interleukins (*IL1A, IL1β, IL4, IL6, IL10, IL11, IL12B, IL17A, IL17F, IL18, IL20, IL21, IL22, IL23A, IL25, IL31, IL33*) and transcription factors *IRF3*, interferon regulatory factor 3; *GLIS1*, Glis Family Zinc Finger 1; *STAT1* and *STAT3*, signal transducers and activators of transcription 1 and 3), as well as transcription corepressor involved in the negative regulation of DNA-binding transcription factor activity (HR, lysine demethylase and nuclear receptor corepressor), vascular endothelial growth factor A (*VEGF-A*), and intercellular adhesion molecule (*ICAM1*). The expression changes between lesional and nonlesional skin of psoriatic patients were assessed before the beginning of apremilast treatment (*Figure 2*, left column). Then, the changes of gene expression were measured in lesional skin after 26 weeks of apremilast treatment against the background of initial measurement prior to the onset of the therapy (*Figure 2*, right column).

An increase in fold changes of gene expression were found in lesional vs. nonlesional skin for *IFNγ, IL1β, IL12B, IL17A, IL17F, IL20, IL21*, and *IL22*. Fold changes of gene expression in lesional skin were elevated for the genes *STAT1* and *IL23A* and reduced for *GLIS1,* but were less pronounced. Our results are similar to those of other studies on the pathogenesis of psoriasis [2, 4, 6, 7, 11], highlighting the central role of the cytokines *IL1β* and *IL17* in inflammation in psoriatic lesions and the involvement of *IL12B, IL20, IL21, IL22, IL23A*, and *IFNγ* for inflammatory benefits that are likely mediated by the transcription factors *STAT1* and *GLIS1*. Changes in the fold of gene expression in other studied genes remained virtually unchanged, less than twofold.

Changes in the gene expression profile in lesional skin after apremilast treatment revealed a three to fourfold reduction in the expression of *IFNγ, IL1β, IL20, and IL21*. The expression levels of *IL6, IL17F, IL22* and *IL31* decreased, but less significantly. Comparing the expression pattern of psoriatic skin lesions vs. nonlesional skin and vs. the expression profile of lesional skin after apremilast treatment, a decrease in the expression of all studied cytokine genes was noted, with an initially increased fold change in lesional skin after apremilast treatment. Previously decreased levels of *TNF* and *GLIS1* expression in lesions were elevated after apremilast treatment. If to compare changes in the columns of the *Table 2* and in *Figure 2*, we can note changes in gene expression levels in psoriatic lesions after treatment with apremilast.

The patient sample was further divided into two groups based on the outcome of apremilast treatment (*Table 1*). Fold change data of individual gene expression (RQ) were used for ROC analysis. The differences between the groups were statistically significant only in changes in the expression levels of the *IL1β, IL6* and *IL17F* genes: in the group of patients receiving apremilast. The best outcome (delta PASI>75%) was a five- to eleven-fold decrease in RQ of these genes vs. the group of other patients.

**Table 2. Median gene expression fold change in psoriasis lesions before the therapy, and at lesions undergo apremilast therapy.**

| *Gene* | *In lesion vs visually clean skin* | *After vs before the therapy* | *ROC analysis AUC and P-value* |
|---|---|---|---|
| *IFNY* | 4.46 (0.09-15.7) | 0.3 (0.02-3.6) | 0.868 (P<0.001) |
| *IRF3* | 0.6 (0.1-1.8) | 0.9 (0.15-5) | 0.667 (P=0.153) |
| *GLIS1* | 0.35 (0.006-2.7) | 1.6 (0.005-176) | 0.785 (P=0.004) |
| *HR* | 1.1 (0.5-3.2) | 0.9 (0.3-10.2) | 0.597 (P=0.426) |
| *STAT1* | 2.5 (0.6-5.4) | 0.6 (0.1-3.2) | 0.868 (P<0.001) |
| *STAT3* | 1.6 (0.6-2.4) | 0.7 (0.3-2.7) | 0.813 (P=0.001) |
| *VEGFA* | 0.86 (0.3-1.8) | 1 (0.3-2.5) | 0.521 (P=0.87) |
| *ICAM1* | 0.98 (0.26-1.7) | 0.75 (0.1-2) | 0.563 (P=0.601) |
| *TNF* | 0.96 (0.16-2.3) | 1.6 (0.1-20.8) | 0.5 (P=1) |
| *IL1A* | 1.2 (0.4-4.1) | 0.7 (0.07-2.9) | 0.722 (P=0.033) |
| *IL1B* | 5.8 (3-24) | 0.3 (0.02-13.7) | 0.951 (P<0.001) |
| *IL4* | 0.9 (0.016-4.4) | 1.3 (0.04-6.2) | 0.611 (P=0.366) |
| *IL6* | 1.5 (0.2-7.2) | 0.5 (0.02-13.6) | 0.681 (P=0.109) |
| *IL10* | 1.5 (0.5-3.2) | 0.6 (0.16-2.3) | 0.819 (P<0.001) |
| *IL11* | 0.6 (0.2-1.8) | 0.9 (0.09-16) | 0.618 (P=0.316) |
| *IL12B* | 12 (0.4-185) | 0.9 (0.03-27) | 0.855 (P<0.001) |
| *IL17A* | 26.7 (1.5-563.4) | 0.6 (0.001-6.4) | 0.958 (P<0.001) |
| *IL17F* | 23 (9.2-65) | 0.5 (0.004-3.8) | 1 (P<0.001) |
| *IL18* | 0.6 (0.5-10.5) | 1.2 (0.09-5) | 0.694 (P=0.114) |
| *IL20* | 13 (0.3-255) | 0.4 (0.003-3.4) | 0.938 (P<0.001) |
| *IL21* | 24.8 (1-207) | 0.2 (0.01-11.6) | 0.979 (P<0.001) |
| *IL22* | 10 (4.4-233) | 0.5 (0.0003-8.4) | 0.979 (P<0.001) |
| *IL23A* | 2.9 (0.4-8.9) | 0.6 (0.15-8.6) | 0.826 (P<0.001) |
| *IL25* | 1.9 (0.3-6) | 1.4 (0.16-2019.8) | 0.535 (P=0.776) |
| *IL31* | 0.9 (0.004-10.8) | 0.5 (0.001-342.6) | 0.603 (P=0.414) |
| *IL33* | 1.9 (0.5-2.9) | 1.2 (0.4-5.6) | 0.611 (P=0.348) |

**Discussion**

It is well known that IL1β enhances *IFNγ* production and acts as a Th17 differentiation factor through direct stimulation. *IL1β* has also been shown to indirectly promote Th17 differentiation through dendritic cell (DC) activation [23]. Activated conventional DCs stimulate the differentiation of naïve T cells into Th17 cells by producing the cytokine *IL17F*, which, along with *TNFα* and *IFNγ*, activates keratinocyte proliferation, angiogenesis and, also initiates an inflammatory cascade in psoriatic lesions [2, 7]. IL1β activity is primarily controlled by the regulation of caspase-1, which itself remains inactive until inflammasome assembly occurs; *IL1β* can also be truncated by neutrophil elastase, proteinase-3, and cathepsin G. Proteases secreted by neutrophils, which are present in psoriatic plaques, were shown to truncate immature *IL1* family cytokines, including *IL1α*, *IL1β*, and *IL36*, thereby potentially enhancing the inflammatory milieu once they are in the skin [23]. Since *IL1β* plays a critical role in the inflammatory cytokine cascade in psoriasis, it is conceivable that the outcome of apremilast treatment associated with *IL1β* expression may be based on differential protease activity due to individual genetic variations. On the other hand, apremilast can inhibit the activation of pronounced proteolysis of immature *IL1β* [24, 25].

IL6 was shown to promote keratinocyte proliferation through Th17 differentiation and production of cytokines (*IL17, IL22, TNF*) that support inflammation in lesions [30]. IL-17F is the most homologous cytokine to *IL-17A*; it induces the expression of several proinflammatory cytokines (*TNF, IL-1b, IL-6*, etc.) and a number of chemokines, and also induces tissue remodeling MMPs and stimulates the production of AMPs. The canonical target of *IL-17* is *IL-6*, the production of which is increased when *IL-17A* acts synergistically with *TNF*, which also leads to the production of *IL-1β* and *IL-8* [31].

To summarize, we can say that the clinical outcome of apremilast treatment depends on the combination of gene expression of the proinflammatory cytokines *IL1β* and *IL17F* and the level of expression of the proinflammatory cytokine *IL6*. These cytokines act synergistically to drive the Th1-type inflammation pathway, which was shown to be a key pathway in psoriatic inflammation. Considering the role of these cytokines in the pathogenesis of psoriasis, it can be assumed that the result of treatment with apremilast is associated with inhibition of the Th1-type inflammation pathway. The best clinical outcome of apremilast use coincides with predominantly reduced expression of *IL1β, IL6*, and *IL17F* genes during psoriasis therapy. This finding also supports the hypothesis that the outcome of apremilast treatment depends on individual patient characteristics.

A study of proteomic cytokine levels in skin biopsies from the same patients showed a positive correlation between *IL1β* and *IL6* levels and PASI score at 26 weeks of therapy, revealing a similar pattern of influence of these cytokines on the outcome of apremilast treatment [32]. Thus, the proteomic predictors and gene expression cytokines influencing the outcome of apremilast treatment are essentially the same, albeit not entirely overlapping. The obtained results are the first step towards developing a predictive model to personalize the use of apremilast in the treatment of severe psoriasis.

**Conclusion**

The patterns of cytokine gene expression profiles are described both for the lesional and nonlesional skin of psoriatic patients. We revealed the leading force of *IFNγ, IL1β, IL12β, IL17A, IL17F, IL20, IL21, IL22,* and *IL23A* cytokines, along with *GLIS1* and *STAT1* transcription factors, in psoriatic lesion formation, thereby confirming Th1/Th17/Th23 proinflammatory axis of psoriasis pathogenesis. We also showed that apremilast treatment reduced the production of all formerly hyperexpressed cytokine genes in psoriatic lesions.

The best clinical outcome was established for the patients with mainly reduced fold change of gene expression of *IL1β, IL6,* and *IL17F* in psoriatic lesions after apremilast treatment.

**Acknowledgments**

The authors are grateful to all patients enrolled as subjects in this study. The research was supported by the Russian Scientific Foundation (project # 18-15-00372).

**Conflict of interest**

The authors report no conflicts of interest pertaining to the study.

**Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the State Research Center of Dermatovenereology and Cosmetology of the Russian Federation Ministry of Healthcare (protocol # 4, 27 Apr 2018).

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