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Inflammation Research

Official Journal of: The
International Association of
Inflammation Societies + The
European Histamine Research
Society

ISSN 1023-3830

Inflamm. Res.
DOI 10.1007/s00011-011-0357-
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Genome-wide gene expression study indicates the anti-inflammatory effect of polarized light in recurrent childhood respiratory disease

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Received: 15 March 2011 / Revised: 16 June 2011 / Accepted: 20 June 2011
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Abstract

Objectives The clinical and molecular effects of whole-body polarized light treatment on children suffering from recurrent respiratory infection were studied.

Methods The incidence and duration of respiratory symptoms as well as the length of appropriate antibiotic therapy were measured. Simultaneously, the genome-wide gene expression pattern was examined by whole genome cDNA microarray in peripheral lymphocytes of children.

Results Twenty of 25 children showed a marked clinical improvement, while in five of 25 had poor response or no changes. The gene expression pattern of the patients' peripheral lymphocytes was compared in *favorable* and *poor* responders. The lymphocytes of the children with a documented improved clinical response to polarized light therapy showed a decrease in the expression of chemokine genes, such as CXCL1, CXCL2, CXCL3, and IL-8, and in that of the TNF α gene. On the contrary, a rapid elevation was found in the expression of the gene encoding for CYP4F2, a leukotriene B4-metabolizing enzyme. In children with poor clinical response to polarized light therapy, no similar changes were detected in the gene expression pattern of the lymphocytes.

Conclusions The improved clinical symptoms and modified gene expression profile of lymphocytes reveals an anti-inflammatory effect of whole-body polarized light irradiation.

Keywords Inflammation · Respiratory symptoms · Polarized light · Gene expression · Chemokine · Leukotriene B4

Introduction

In-vivo and in-vitro data [1–3] demonstrated that polarized light has a selective effect on the various cells of the immune response, as well as other biological model systems, in contrast to diffuse visible light of the same wavelength range and power density used as control. The experiments [2] proved that polarized light has a significant effect on the gene and protein expression of interleukin-6 (IL-6), a central regulatory molecule of acute inflammation, reducing IL-6 production in circulating blood monocytes while increasing that in human B lymphocytes. This fact raises the possibility that polarized light might play a selective, cell-specific role in the regulation of acute and chronic inflammatory reactions. The effect of polarized light seems to belong to the wide variety of environmental epigenetic effects influencing immune homeostasis.

Besides an increase in absolute lymphocyte count, a recently published in-vivo study [3] also confirmed a significant elevation in the number of CD3⁺ CD4⁺ T lymphocytes. This seems to support also the early findings by Fenyő [4] that polarized light treatment evoked an increase in the local T-lymphocyte count in wounded skin during the healing process [4]. Numerous additional studies

Responsible Editor: Michael Parnham.

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confirm earlier results [5, 6] by proving the accelerating effect of polarized light on wound healing. Well-established clinical observations have demonstrated the effect of polarized light on the significant acceleration of epithelialization, increase in collagen production, and the reorganization of the extracellular matrix [7]. Positive results have been reported recently concerning the application of polarized light in treating keratosis [8]. Research conducted with molecular and immunological methods also shows that polarized light selectively stimulates the cell division of fibroblasts and biosynthesis of collagen [9]. The significant beneficial effect of polarized light has also been described in relation to carpal tunnel syndrome neuropathy, where over 90% of the patients experienced a significant reduction in pain and abnormal sensations [10]. The antidepressant effect of light is also well-known, especially in countries with a particularly extended winter season [11].

In the present research, the clinical and immunological effects of in-vivo whole-body polarized light treatment was studied on the peripheral lymphocytes of children suffering from persistent respiratory diseases, with a favorable (20 of 25) or poor (5 of 25) clinical response. The clinical findings clearly suggest a marked improvement in the condition of recurrent respiratory patients. In the present work we used a genome-wide gene expression approach to follow the changes in the gene expression pattern of peripheral lymphocytes. The results were validated by RT-PCR technology. In addition to the conventional bioinformatic procedures, gene set enrichment and genetic pathway analyses were applied.

Parallel to clinical improvement, the genomic results suggest that whole-body polarized light treatment causes significant changes in the expression of about 30 genes. In this first stage of the experiment we focused on the set of the annotated genes obviously involved in inflammatory events. Primarily, decreased expression of chemokine genes, TNF α , and elevated expression of a gene encoding for an enzyme responsible for the degradation of leukotriene B4 was found.

None of these significant changes were found in patients who, on the basis of clinical symptoms, respond poorly or not at all to polarized light therapy.

Based on clinical observations and whole genome gene expression studies, evidence is provided for the anti-inflammatory effects of whole-body polarized light treatment in children with recurrent respiratory symptoms.

Patients and methods

Patients

The procedure received official permission from the local ethics committee and the Scientific and Research Ethics Committee of the Medical Research Council of Hungary according to the European standards (Law of Human Genetics, 2008/XXI). Children suffering from recurrent respiratory infection received treatment based on medical referrals and written informed consent of the parents or legal representative. All clinical studies were performed in accordance with Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki and its amendments in Tokyo and Venice.

The open prospective study was carried out with 25 children (11 girls and 14 boys), with a median age of 4 (1–7.8 years of age). Children had a pulmonological examination at the Pediatric Pulmonology Clinic of Szent János Hospital, Budapest. The basis of recruitment was their medical history: children suffering from respiratory diseases (head cold, cough, bronchitis) for more than 7 days per month in the 3 months preceding the examination; their symptoms beginning when they started attending child care facilities, and not previously prone to diseases. The examination found no pathological deviations in their blood count or iron status, serum immunoglobulin (IgA, IgG, IgM) were in the range appropriate for their age, and chest X-ray showed no

Table 1 Clinical parameters of children suffering from recurrent respiratory disease and responding favorably to polarized light therapy

Respiratory symptoms				Antibiotic therapy			
5 weeks preceding treatment <i>n</i> = 17 ^a		5 weeks of treatment <i>n</i> = 20 ^b		5 weeks preceding treatment		5 weeks of treatment	
Cases	Duration (days)	Cases	Duration (days)	Cases <i>n</i> = 25	Duration (days) <i>n</i> = 13	Cases <i>n</i> = 25	Duration (days) <i>n</i> = 3
1.7	9.4	1.1	4.3	0.6	8.6	0.12	5.6

^a Eight other children had continuous symptoms

^b Five other children had continuous symptoms

infiltrates or pathological deviations suggesting developmental disorders of the respiratory system. Three children had elevated levels of total serum IgE, but specific IgE and/or skin prick tests did not confirm sensitization; the children had no atopic diseases. Exclusion criteria were as follows: lack of necessary cooperation, manifest psychosis, mental disorders, emotional instability, photosensitivity or a primary disease increasing susceptibility to photosensitivity, or taking medication which causes photosensitivity.

The clinical state of patients was monitored by questionnaires. The patients' parents were asked to report on the number of days with symptoms, and those with antibiotic treatment. Results are shown in Table 1.

Progress of the study

Children received 10 treatments with polarized light over a period of 5 weeks. The duration of light irradiation was 10–15 min depending on their height (10 min under 120 cm, 15 min above 120 cm) based on the manufacturer's recommendation. Anticoagulant-treated venous blood samples were collected: (1) before the first treatment (as starting value), (2) 20 min after the first treatment (immediate response), and (3) before the tenth treatment at the 5th week (delayed response). Gene expression analysis was performed on the mRNA of the isolated peripheral blood mononuclear cells of nine children (lymphocyte percentage >97%): the five children who showed the best response, and the four children who showed the least clinical response to polarized light treatment.

Light source

The patients were irradiated using the Sensolite[®] device (Polarium Ltd., Hungary), suitable for treating the whole-body. In the Sensolite device, the polarized light is generated with the help of powerful light-emitting diodes (LEDs). All the LEDs radiate in the warm white and near-infrared (IR) range and the inner surfaces (closer to the light source) of the Plexiglas sheets are covered by a polarizing filter. A lens (only for warm white LEDs) narrows the light source. No UV component is produced during radiation. Light sources: warm white LED, IR LED, with a wavelength of 850 nm. Light power density: 40–60 mW/cm²

Microarray studies on gene expression

Peripheral blood mononuclear cells were isolated by conventional Ficoll/Hypaque gradient from the anticoagulant-treated venous blood of five children responding very well and four children not responding to irradiation with polarized light, using gradient centrifugation and following

red blood cell depletion. Cell purity was checked by immunophenotyping. Total RNA was isolated from a cell suspension containing 2×10^7 white blood cells using the Qiagen miRNeasy kit, following the manufacturer's directions. RNA quality was checked with the Agilent 2100 Bioanalyzer, and one-color labeling was performed using the Agilent QuickAmp Labeling kit. The Agilent Whole Human Genome 4 × 44 K slide was used for microarray examination. The slides were scanned with the Agilent microarray scanner. Data were extracted using Agilent Feature Extraction Software. All microarray data were validated by RT-PCR analysis, performed by commercially available primers.

Statistical methods

Statistic analysis of the microarray analysis and RT-PCR validation was performed by GeneSpring GX 11.0 software and Student's *t* test. Further statistical and bioinformatic analyses were done with the help of Gene Set Enrichment Analysis (GSEA), Molecular Signatures Database (MSigDB) and Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Inc.) software.

Results

Clinical findings

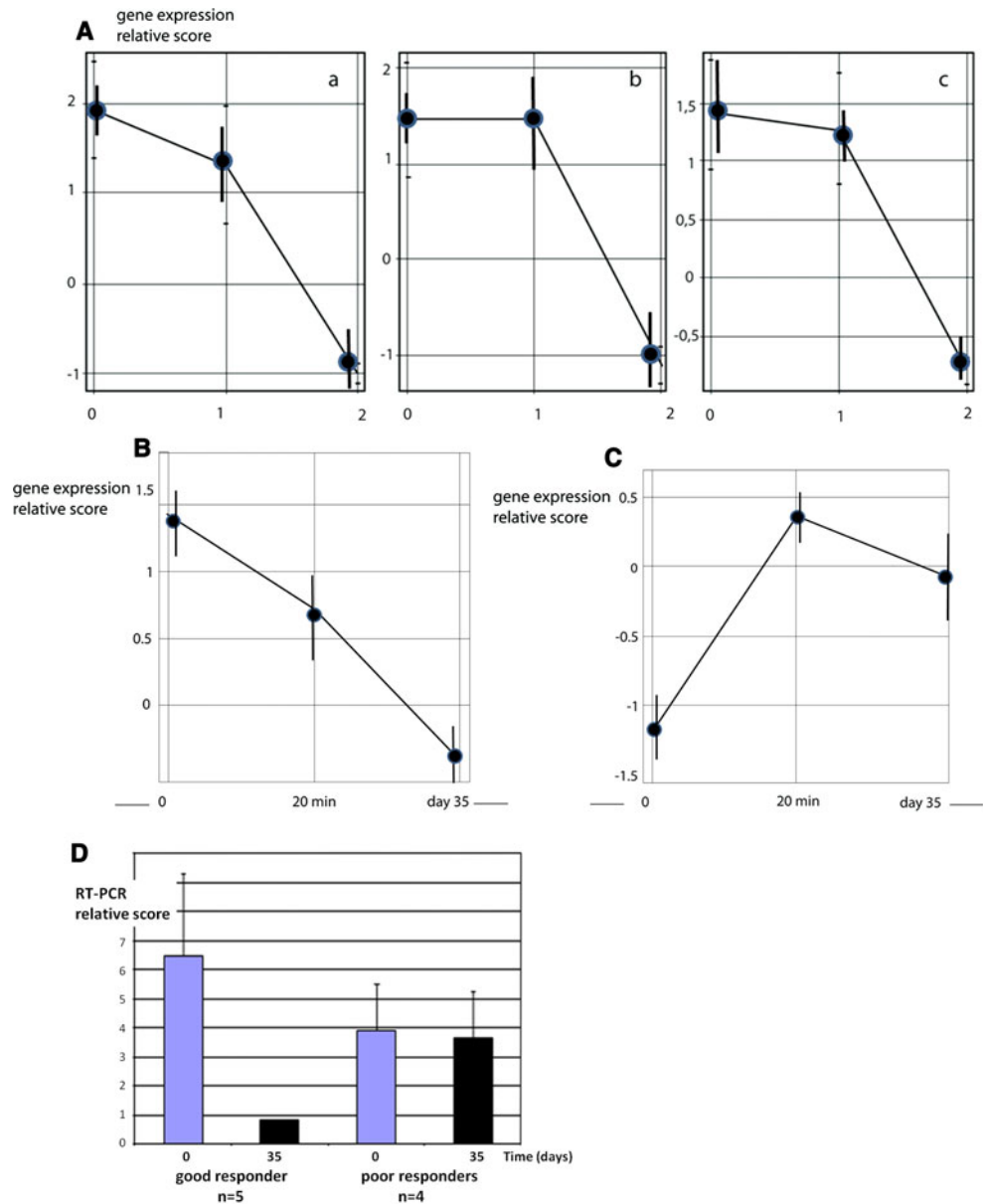
The clinical data from the 5 weeks preceding whole-body polarized light treatment were compared with the data collected during the 5 weeks of treatment (Table 1). In the 5 weeks preceding treatment, 17 children experienced respiratory symptoms an average of 1.7 times, with an average duration of 9.4 days. Eight children had continuous symptoms. In the 5 weeks of treatment the average number of cases dropped to 1.1, with an average duration of 4.3 days. Of the eight children who had complained of continuous symptoms, five continued to experience respiratory symptoms on a daily basis. Thirteen children required antibiotic therapy in the 5 weeks preceding the light therapy (for an average duration of 8.6 days), which means an average of 0.6 cases calculated for 25 children. In the 5 weeks of light therapy only three children required antibiotic treatment (for an average duration of 5.6 days), corresponding to an average of 0.12 cases calculated for 25 children. Whole-body polarized light treatment resulted in a complete absence of symptoms in five of the 25 children, while fifteen children showed a large improvement in clinical symptoms. This was indicated by a decrease in the number and duration of diseases, as well as a reduction in the use of antibiotic treatment. In five children the

Table 2 Genes with significantly modified expression in the lymphocytes of the five children with a favorable clinical response to polarized light therapy

Marked genes with modified gene expression	Known gene function	Direction of change in gene expression	Fold change	<i>p</i>
<i>CXCL2</i>	Chemokine	↓	7.17	<0.020
<i>CXCL1</i>	Chemokine	↓	6.41	<0.014
<i>CXCL3</i>	Chemokine	↓	5.46	<0.014
<i>IL-8</i>	Chemokine	↓	4.84	<0.013
<i>TNFα</i>	Chemokine	↓	3.06	<0.023
<i>IER3</i>	“Early response” protein	↓	2.59	<0.021
<i>SOD2</i>	Dismutase	↓	2.07	<0.005
<i>KLF5</i>	Differential transcription factor	↓	2.12	<0.021
<i>CYP4F2</i>	Leukotriene B4 metabolizing enzyme	↑	2.51	<0.014
<i>KLK3</i>	Prostate-specific antigen	↑	1.85	<0.003
<i>LRP3</i>	LDL receptor-like protein	↑	1.85	<0.003
<i>CACNA1E</i>	Voltage-dependent Ca channel	↑	2.15	<0.002
<i>KRTAP2-4</i>	Keratin-associated protein	↑	1.75	<0.002

n = 5

Fig. 1 Gene expression pattern of peripheral lymphocytes from children with beneficial clinical response to whole-body polarized light therapy. Of the significantly changing genes, there are three chemokines, CXCL2, CXCL1 and IL-8 (A, a,b,c, respectively), TNF α (B) and a leukotriene metabolizing enzyme (CYP4F2) (C) collected at three different time points (0, 1, 2: at 0 min, at 20 min, and before the last treatment at the 35th day, 5th week, respectively) (27,000 human genes, 40,000 tests, Agilent 40 K). Gene expression is expressed in relative scores. D RT-PCR validation of the TNF α results, comparing the changes in TNF α mRNA from lymphocytes of patients with favorable ($n = 5$) and poor or no ($n = 4$) clinical response to whole-body polarized light treatment



continuous symptoms observed before the treatment failed to improve as a result of the therapy.

Gene expression by whole genome gene expression and RT-PCR

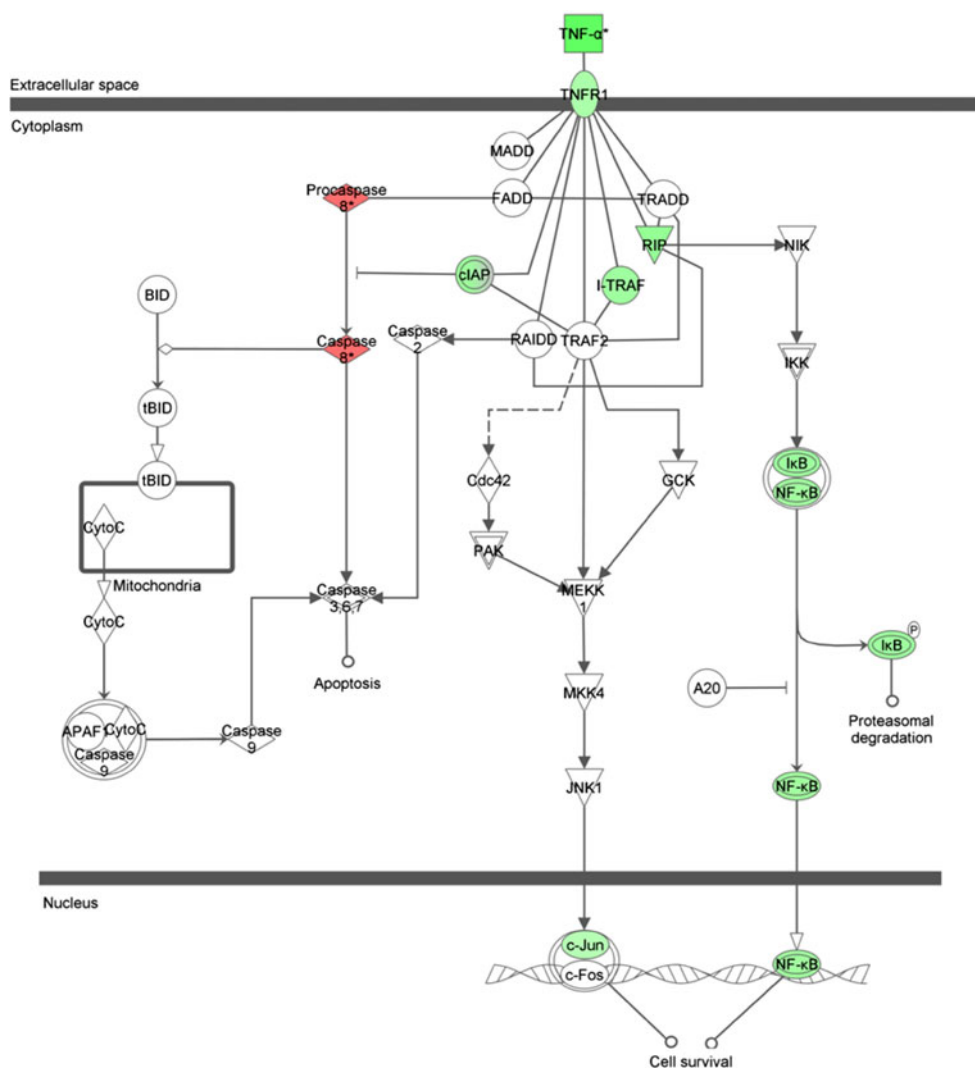
Polarized light treatment is followed by changes in a number of genes in the peripheral blood lymphocytes. Twenty-eight genes can be identified which change significantly and show at least a significant increase or decrease compared to the starting values. The annotated genes, the fold change and the p values are shown in Table 2.

Figure 1a shows the gene expression of three chemokines, CXCL2, CXCL1 and IL-8, TNF α (Fig. 1b)

decreases, and increases in a leukotriene metabolizing enzyme (CYP4F2, Fig. 1c) in peripheral lymphocytes collected at three different time points (at 0–20 min, after the first treatment, and at the 5th week, after ten treatments).

Gene expression of main chemokines decreased uniformly in all five children who showed a favorable clinical response to polarized light therapy. Validation with RT-PCR procedures confirmed the microarray data. Figure 1d shows that while the TNF α mRNA values are significantly decreased in the lymphocytes of children with favorable clinical response after ten light treatments, those from poor or no responders do not change. Interestingly the patients with a subsequent good response to polarized light treatment have a significantly higher baseline TNF α expression

Fig. 2 Gene network and pathway analysis (IPA analysis) related to TNF α signaling (green inhibition, red stimulation)



compared to that from children with poor or no clinical improvement.

The complete analysis of the TNF α pathway clearly shows the inhibition of the NF κ B signaling path (in green) (Fig. 2), based on the IPA of the gene expression pattern of children with a good clinical response to polarized light therapy.

Concerning the other affected pathways, the preliminary gene expression data suggest that the natural immune response and the apoptosis gene group are most affected in lymphocytes by irradiation with polarized light (data not shown).

In contrast with the above, in the significantly lower number of children (5 of 25) whose clinical condition did not improve as a result of whole-body polarized light therapy, there was no change in the gene expression of chemokines, TNF α and CYP4F2. At the level of the whole genome, we observed a significant reduction, however, in

the expression of two genes and a significant increase in the expression of four (Table 3). Of the former, the decrease in the expression of GCC2, a brefeldin A sensitive peripheral membrane protein of the trans-Golgi network, and a late cornified envelope protein (LCE2D) gene warrants further research.

Discussion

Recurrent respiratory diseases of children attending child care facilities in infancy and early childhood constitute a significant part of the workload of family doctors in the autumn and winter months [12]. These diseases are free of complications and do not cause permanent damage, but they place a serious burden on families and on the health care system due to the great number of patients. Since these children are basically healthy, they become free of

Table 3 Changes in gene expression in the lymphocytes of children with poor or no clinical response to polarized light therapy

Significantly changing genes	Known gene function	Direction of change in gene expression	Fold change	<i>p</i>
<i>GCC2</i>	GRIP and coiled-coil domain containing 2	↓	2.66	<0.041
<i>LCE2D</i>	Late cornified envelope 2D	↓	2.05	<0.047
<i>TGFBR3</i>	Transforming growth factor, beta receptor III	↑	1.04	<0.012
<i>KLRK1</i>	Killer cell lectin-like receptor subfamily K, member 1	↑	1.14	<0.012
<i>PRKAG1</i>	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	↑	1.06	<0.040
<i>C19orf31</i>	Chromosome 19 open reading frame 31	↑	1.34	<0.014

n = 4

symptoms if they stop attending child care facilities; however, parents can rarely afford this in the current social conditions [13]. This is why we consider it important to employ a supportive therapy which helps to prevent the development of the disease, without side effects.

Earlier results [2] suggested that polarized light applied in vitro (in contrast with similarly used diffuse light) enhanced immunoglobulin M (IgM) synthesis in a human B cell line due to an increased local IL-6 production and autocrine effect by B cells. At the same time, its effect inhibited significantly IL-6 production in monocytes regulating numerous pro-inflammatory mediators.

This effect suggests that reducing inflammation (i.e., impaired IL-6 production by monocytes) and enhancing the active immune response (i.e., stimulation of B and T lymphocytes, especially CD4⁺ helper T cells) can be an adjuvant strategy in treating certain childhood diseases (e.g., respiratory inflammation). Indirectly (besides the proven and well-known anti-depressant effect of visible and polarized light [14]), a faster recovery from these unpleasant and prolonged childhood conditions has a beneficial effect.

Since polarized light reaches the immunologically active cells circulating in the capillaries of the surface layers of the skin, the possible effect of the polarized light

seems to influence the entire immune response through blood and lymph circulation.

In order to highlight some results among these findings, we observed a very rapid reduction in the level of TNF α mRNA and a slower, but significant, decrease in the expression of four genes of major chemokines, CXCL1, CXCL2, CXCL3 and IL-8, which play a key role in the respiratory inflammation response [15]. Interestingly a very fast elevation (even after the first treatment) in the gene expression of the pro-inflammatory leukotriene B4 degrading omega-hydroxylase enzyme (CYP4F2) was detected. On the basis of several pathway analyses, 31 of the 384 known gene ontology pathways were found to be significantly affected (detailed results not shown here). The presentation of a representative biological pathway of TNF α also confirms the conclusion that whole-body polarized light therapy inhibits the NF κ B signaling system [16]. These results support the results reporting clinical improvement.

In contrast, in the significantly lower number (5 of 25) of children whose clinical condition was not improved by whole-body polarized light therapy, there were no changes in the activity of these genes. In the lymphocytes of the four children examined without a clinical response, microarray examination showed a significant reduction in

the expression of two genes (GCC2, a brefeldin A sensitive membrane protein of the trans-Golgi network, and a late cornified envelope protein LCE2D) as well as an increase in the expression of four others at the level of the whole genome. As far as the mechanism of the action of linearly polarized light on lymphocytic gene expression is concerned, one may speculate on the change in membrane dynamics of lipid rafts. Further in-vitro and model studies are in progress to uncover the mechanism.

Natural and artificial light seems to be a newly recognized set of the epigenetic factors which deserves greater attention and more detailed molecular studies [17]. Our results indicate that whole-body polarized light therapy can become an effective supportive tool in treating inflammatory diseases, in relieving and perhaps preventing their symptoms.

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