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# Expression of Neuropeptides, Neurotrophins, and Neurotransmitters in the Skin of Patients with Atopic Dermatitis and Psoriasis

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Expression of neural marker PGP9.5, amphiregulin, semaphorin-3A, calcitonin gene-related peptide and its receptor, nerve growth factor and its receptor, substance P and its receptor, and expression frequency were analyzed in biopsy specimens from patients with atopic dermatitis and psoriasis and healthy volunteers by immunohistochemical method. Nerve fibers penetrated into the epidermis more frequently, and amphiregulin expression was significantly higher in patients with atopic dermatitis and psoriasis comparing to the control. Expression of semaphorin-3A in patients with atopic dermatitis was less frequent than in the control. These findings reflect the involvement of amphiregulin and semaphorin-3A in the improvement of skin innervations and penetration of nerve fibers into the epidermis, and due of which these proteins mediate the development of itch.

**Key Words:** *atopic dermatitis; psoriasis; itch; amphiregulin; semaphorin-3A*

In patients with skin diseases, itch is one of the most annoying symptoms leading to the development of insomnia, anxiety, and depression. Itch can accompany inflammatory process in some skin diseases and serves as one of the main diagnostic symptoms of atopic dermatitis (AD).

Development of itch during chronic inflammatory skin diseases is related to the effects of itch transmitters, *e.g.* histamine, proteases, neuropeptides (substance P), cytokines (IL-31), on pruriceptive nerve endings of sensory C-fibers [4,6,7]. Quantitative and qualitative parameters of the nerve network in the skin determine itch threshold in patients with chronic inflammatory dermatosis. The increase in the number of sensory nervous endings in the skin and high sensitivity of nerve endings to prurigenous factors promote a decrease in itch threshold. This effect is named nerve sensitization and is typical of patients with AD. The participation of sensory nervous fibers in itch development is not confined to pruriception. They can release bioactive substances mediating the development of inflammation [3,11,14], *e.g.* neuropeptides substance P and calcitonin gene-related peptide (CGRP) promote the development of inflammatory process in the skin and itch [12,15].

The density of nerve endings in the skin is regulated by factors of nerve growth and reduction. Nerve growth factor (NGF) and epidermal growth factor amphiregulin promote the growth of nerve endings [2,8]. On the contrary, factor of nerve reduction semaphorin-3A negatively affects the growth of nerve fibers [5].

Here we studied the expression of factors stimulating growth of nerve fibers (NGF and amphiregulin), factors for nerve reduction (semaphorin-3A), and neurotransmitters involved in itch development (substance P and CGRP) in the damaged skin of patients with AD and psoriasis.

## MATERIALS AND METHODS

Thirty patients with AD (14 women and 16 men aging 18-43 years) and 30 patients with psoriasis (9 women

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and 21 men aging 21-68 years) took part in the study. Skin biopsies were taken from affected areas. Skin biopsies from 10 healthy volunteers were used as the control.

Biopsy samples were processed routinely: fixed in 10% buffered formalin, dehydrated in isopropyl ether (Leica ASP300 automatic vacuum system), saturated with paraffin, and embedded in paraffin blocks; 5-μ sections were sliced using a Leica RM2125RT rotation microtome, then mounted on polylysine-precoated slides, and exsiccated in a thermostat at 36°C for 12 h.

Expression of nervous fiber markers PGP9.5, amphiregulin, semaphorin-3A, CGRP and its receptor (CGRP-R), NGF and its receptor (TrkA), and substance P and its receptor in skin structures was estimated using immunoperoxidase immunohistochemical method [1].

Rabbit polyclonal antibodies to PGP9.5 (No. 1213010A; Cell Marque), rabbit polyclonal antibodies to semaphorin (No. 251491), CGRP (No. 250602), CGRP-R (No. 254502), substance P (No. 250865) and its receptor (No. 250870; all these antibodies were manufactured by Abbiotec), murine monoclonal antibodies to amphiregulin (No. sc-74501; Santa-Cruz Biotechnology), rabbit monoclonal antibodies to NGF (clone EP1320Y) and TrkA (clone EPR1104(2); Epitomics), and universal peroxidase-containing detection system (Histofine Simple Stain MAX PO (MULTI); Nichirei Biosciences Inc.) with universal immunoperoxidase polymer were used for immunophenotyping. Visualization of the reaction was performed using DAB-chromogen.

Immunohistological reaction was conducted in accordance to manufacturer's instruction. Sections were boiled in citrate buffer (pH 6.0) in a microwave at maximal capacity of 900 W (3×7 min with 1-min intervals) for antigen retrieval. After cooling, the samples were washed with phosphate buffer containing Twin-20 (pH 7.6). The sections were treated with 0.3% $H_2O_2$ in methanol (1:1) for blockade of endogenous peroxidase activity and incubated with 5% BSA for 60 min before treatment with primary antibodies. Incubation with primary antibodies was conducted at 23-25°C for 60 min and with secondary antibodies at 23-25°C for 30 min.

After immunohistochemical staining, background contrasting of the sections with Mayer's hematoxylin was performed. The obtained immunohistochemical samples were placed under coverslips and examined under a Leica DM4000B light microscope with Leica DFC320 digital camera.

Localization and frequency of expression of NGF and its receptor TrkA, substance P and its receptor, CGRP and CGRP-R, amphiregulin, and semaphorin-3A in the skin of patients with AD and psoriasis were estimated.

Statistical analysis of obtained data was performed using $\chi^2$ test. Analysis was conducted using Statistica 10.0 software.

## RESULTS

In all patients with AD and psoriasis and healthy volunteers, protein PGP9.5 was expressed on nervous fibers near the sweat glands and in neurovascular bundles located between smooth muscle bundles of arrector pili muscles, and nerves of the derma. Fine $PGP9.5^+$ nerve fibers were found in the papillary dermis. Ingrowth of $PGP9.5^+$ nerve fibers into the epidermis was observed in all patients with AD and psoriasis, while in the control, no $PGP9.5^+$ nerve fibers were found in the epidermis (Fig. 1).

Expression of amphiregulin was found in the cytoplasm of epidermal keratinocytes, epithelial cells of the outer and inner root sheath, and epithelial cells of sweat glands in 26 patients with AD and 27 patients with psoriasis. Expression of amphiregulin in the skin of patients with AD and psoriasis was found more frequently ($p<0.05$) than in the control (3 volunteers; Table 1).

Expression of semaphorin-3A was observed primarily in the basal and suprabasal layers of the epidermis in 6 patients with AD, 17 patients with psoriasis, and 7 healthy volunteers. The frequency of semaphorin-3A expression in patients with AD was significantly lower than in the control ($p<0.05$).

Expression of CGRP was found in fine nerve fibers between keratinocytes in 3 patients with AD and 6 patients with psoriasis, but was not observed in the control (Table 1). CGRP-R was also expressed in the fine nerve

**TABLE 1.** Frequency of Expression (%) of Neuropeptides, Neurotrophins, and Neurotransmitters in the Skin of Patients with AD and Psoriasis

| Neuropeptides, neutrophins, and neurotransmitters | Control (*N*=10) | Patients with AD (*N*=30) | Patients with psoriasis (*N*=30) |
|---|---|---|---|
| Protein PGP9.5 | 0 | 100* | 100* |
| Amphiregulin | 30.0 | 86.7* | 90.0* |
| Semaphorin-3A | 70.0 | 20.0* | 56.7 |
| CGRP | 0 | 10.0 | 20.0 |
| CGRP-R | 0 | 10.0 | 20.0 |
| NGF | 80.0 | 100 | 100 |
| NGF receptor TrkA | 0 | 23.3 | 26.7 |
| Substance P | 0 | 16.7 | 16.7 |
| Receptor to substance P | 0 | 26.7 | 10.0 |

**Note.** *$p<0.05$ in comparison with the control.

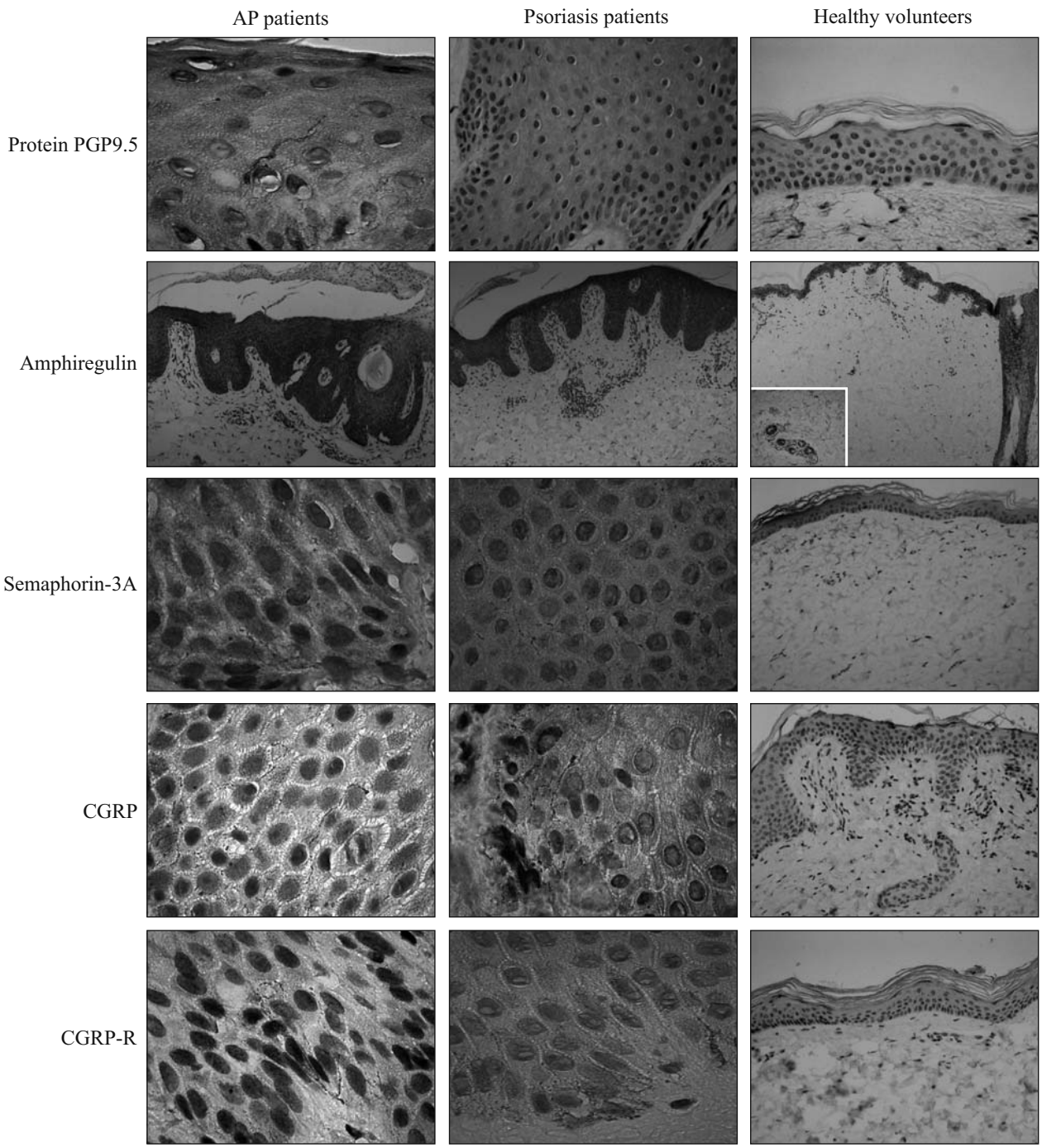


**Fig. 1.** Expression of protein PGP9.5, amphiregulin, semaphorin-3A. CGRP, and CGRP-R in the skin of patients with AD, psoriasis, and in the skin of healthy volunteers. Here and in Fig. 2: immunohistochemical analysis with monoclonal and polyclonal antibodies.

fibers between keratinocytes in 3 patients with AD and 6 patients with psoriasis. In some sections, fine fibers expressing CGRP and CGRP-R were melanocyte processes of from the basal layer of the epidermis (Fig 1). Expression of CGRP-R was not observed in the control.

Expression of NGF was found in the cytoplasm of keratinocytes in the epidermis and epithelial cells of outer and inner root sheath, and in epithelial cells of sweat glands in all patients with AD and psoriasis. In the control, NGF expression was found in 8 volunteers. Expression of NGF receptors (TrkA) was observed on the membrane of keratinocytes in the epidermis (Fig. 2) in 7 patients with AD and 8 patients with psoriasis, but in none of the controls.

Expression of substance P was found in fine fibers between keratinocytes, which probably were melano-

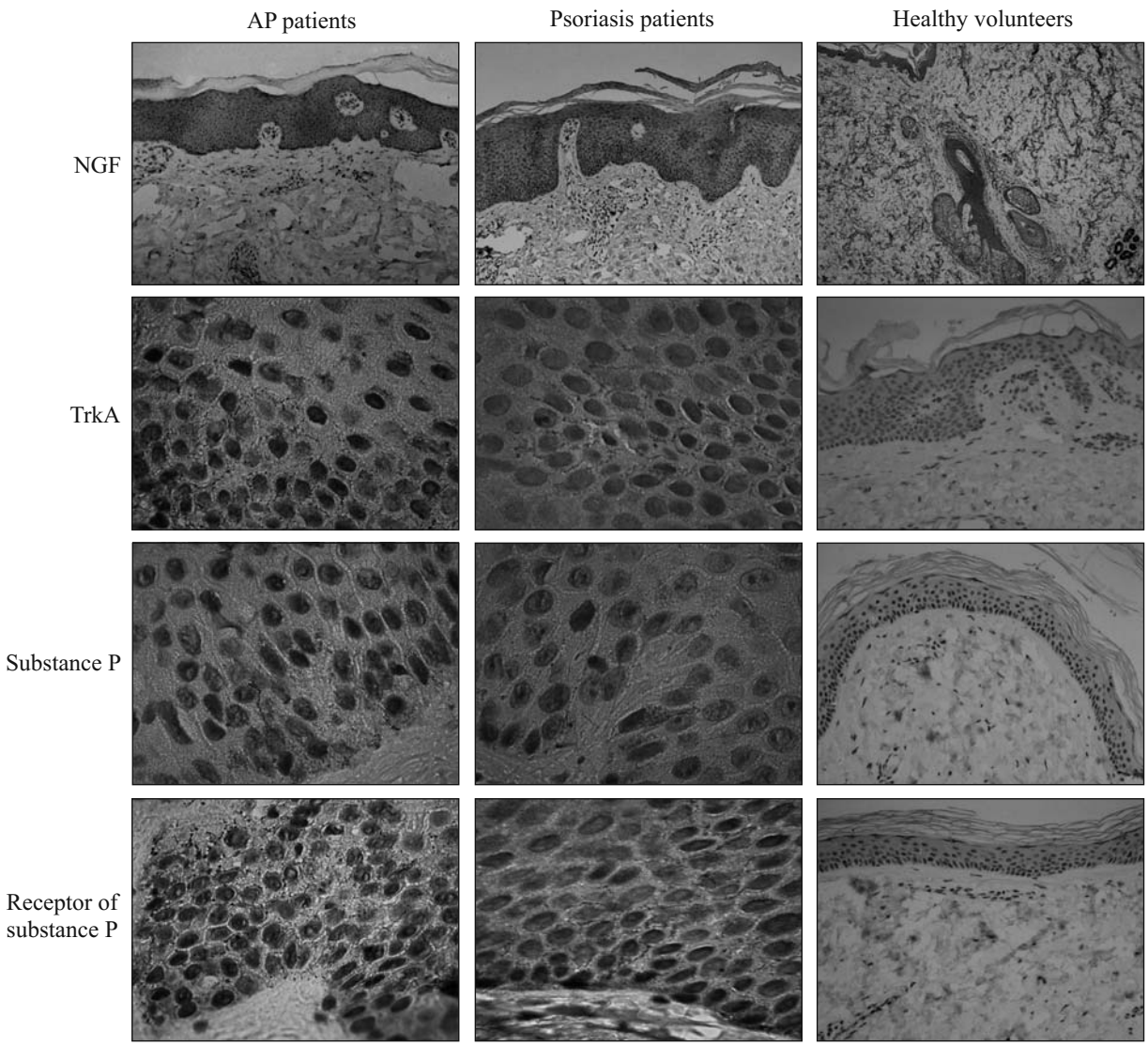


**Fig. 2.** Expression of NGF and its receptor TrkA, and substance P and its receptor in the skin of patients with AD, psoriasis, and in the skin of healthy volunteers.

cyte processes, in 5 patients with AD and 5 patients with psoriasis, but was absent in the control. Expression of receptor to substance P was observed in keratinocyte membranes in the epidermis of 8 patients with AD and 3 patients with psoriasis, and was absent in the control (Table 1).

Disturbances of T-cell immunity play a pivotal role in the pathogenesis of inflammatory diseases of skin, *e.g.* AD and psoriasis. However, peripheral nervous system can affect the development of inflammatory process mediated by Th1 cells in AD and Th2 cells in psoriasis [8,13].

Our study shows that nervous fibers penetrate in the epidermis during AD and psoriasis. Enhanced sensitivity to itch serves as clinical relevance of an increase in the density of nerve fibers in the skin [3]. Increased density of nerve fibers expressing neuropeptides, substance P, and CGRP was found in patients with AD [6,9,12]. Enhanced growth of nerve C-fibers mediating itch was also observed in psoriasis [3].

Experiments on cell cultures of dorsal ganglion neurons from the spinal cord show that impaired balance between the factors stimulating the growth of nerve fibers (NGF and amphiregulin) and factors reducing nerves (semaphorin-3A) is the cause for enhanced growth of nerve fibers [5,8]. Our results indicate that this balance can be shifted due to both enhanced production of substances stimulating nerve growth and suppressed production of semaphorin-3A, the factor of nerve reduction.

Thus, the increase in amphiregulin expression and decrease in semaphorin-3A expression in the epidermis of patients with AD and psoriasis indicate the involvement of amphiregulin and semaphorin-3A in

enhancement of skin innervations manifested in penetration of nerve fibers in the epidermis. The observed participation of amphiregulin and semaphorin-3A in skin innervations attests to the role of these substances in the mechanisms of itch development in patients with AD and psoriasis.

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