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INVESTIGATIVE REPORT

Gene Expression Analysis of the Corticotrophin-releasing Hormone-proopiomelanocortin System in Psoriasis Skin Biopsies

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The corticotrophin-releasing hormone-proopiomelanocortin (CRH-POMC) system in the skin coordinates pigmentation and the immune response. The aim of this study was to evaluate the regulatory role of the neuroendocrine system in the pathogenesis of psoriasis. Using quantitative real-time-PCR, mRNA expression levels of 15 genes related to the CRH-POMC system were measured in punch biopsies from lesional and non-lesional skin of patients with psoriasis and from skin of healthy control subjects. Statistically significant up-regulation of POMC, CRH receptor type 1, melanin-concentrating hormone receptor (MCHR1) and melanocortin receptors 2, 3 and 4 mRNA expression in lesional and in non-lesional skin compared with healthy control samples were established. Tyrosinase (*TYR*), *T(Y)RP-1* and *ASIP* genes were statistically significantly down-regulated in lesional and non-lesional skin of psoriasis samples compared with healthy subjects. The up-regulation of POMC, melanocortin receptors, CRH receptor type 1 and MCHR1 in the lesional and non-lesional skin of psoriasis patients supports the importance of the local CRH-POMC system in the pathogenesis of psoriasis. *Key words: psoriasis; CRH-POMC system; mRNA expression.*

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The central nervous system (CNS) regulates inflammatory responses through hormonal and neuronal routes. The neuroendocrine stress response (the hypothalamic-pituitary-adrenal (HPA) axis) and the sympathetic and parasympathetic nervous systems generally inhibit inflammation at the systemic and regional levels, whereas the peripheral nervous system tends to amplify local inflammatory responses (1). A blunted neural regulation is seen in a wide range of autoimmune and inflammatory diseases in humans, such as rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus and irritable bowel syndrome (2, 3).

The presence of cutaneous corticotrophin-releasing hormone (CRH) peptide and corticotrophin-releasing hormone receptor (CRH receptor type 1 and 2 (CRH-R1 and CRH-R2)) peptides, which are the binding site for CRH and the related urocortin peptide, suggests a potential CRH-induced neuroendocrine pathway in the skin (4, 5). Cutaneous expression of the CRH-proopiomelanocortin (CRH-POMC) system is highly organized, encoding mediators and receptors similar to the HPA axis (5, 6) including key enzymes of corticosteroid and glucocorticoid synthesis (6). CRH stimulates CRH-R1 and induces the production and secretion of POMC (7). In melanocytes and fibroblasts CRH-induced CRH-R1 stimulation upregulates POMC expression and production of adrenocorticotropic hormone (ACTH) through the activation of cAMP-dependent pathway(s). Melanocytes respond with enhanced production of cortisol and corticosterone, which is dependent on POMC activity. Fibroblasts respond to CRH and ACTH with enhanced production of corticosterone, but not cortisol, which is produced constitutively (6). The POMC system consists of the melanocortin peptides α-, β-, and γ-melanocyte stimulating hormone (α-, β-, γ-MSH) and ACTH, which derive from post-translational processing of the precursor molecule proopiomelanocortin (POMC); a family of 5 melanocortin receptors (MCRs); 2 endogenous melanocortin receptor antagonists: agouti (ASIP) and agouti-related protein (AGRP) (8). The CRH-POMC system in the skin primarily coordinates pigmentation and the immune response (4, 5).

The expression of the components of the CRH-POMC system in the skin has been detected in different cell types. In the skin CRH mRNA expression has been demonstrated in cultured melanocytes and keratinocytes (5). In humans, CRH-R1 is predominantly expressed in all major skin cell populations (9), while CRH-R2 is expressed in dermal cells including hair follicle keratinocytes, melanocytes and follicular papilla fibroblasts, sebaceous and eccrine glands, muscle and dermal blood vessels (10). CRH-R1 mediates most phenotypic effects of CRH, while the main adnexal location of CRH-R2 indicates a role for this receptor in hair cycling (9). The *POMC* gene is expressed by cutaneous keratinocytes and melanocytes (8). In the skin the highest expression of MC1R mRNA expression has been detected in melanocytes (11). Human MC1R has the highest, and almost equal, affinity for α-MSH and ACTH, less affinity for β-MSH, and least affinity for γ-MSH (12). MC2R is the classical adrenocortical ACTH receptor (8) and its

expression has been detected in keratinocytes (13) and adipocytes (14). MC3R expression in the skin has been shown in our previous studies, although its expression has not been localized to distinct cell types (15). All of the melanocortins are roughly equipotent with MC3R (8). MC4R is expressed in dermal papilla cells (16) and melanocytes (17), and is activated by α-MSH and ACTH (8). The expression of MC5R has been detected in skin mast cells, adipocytes and sebocytes (5, 18). Its affinity for POMC peptides is similar to that of MC1R (8).

Tyrosinase (TYR) is regarded as the key enzyme in melanogenesis; it is found in melanosomes (19). Tyrosinase-related protein 1 (TYRP1) and dopachrome tautomerase (DCT) are additional melanogenesis enzymes. The signal from the CRH-POMC system in human melanocytes reaches the melanogenesis enzymes TYR, TYRP1 and DCT over MC1R through cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway and it is modulated by Wnt and MAPK (mitogen-activated protein kinase) pathways (17, 20). ASIP is an antagonist of human MC1R. It acts as a competitive inhibitor of α-MSH and inhibits the expression of the melanogenic enzymes tyrosinase, TYRP-1, and DCT (21).

In cultured human melanocytes the melanogenic actions of α-MSH were inhibited by melanin-concentrating hormone (MCH), resulting in reduced melanin production in pigment cells. Such evidence suggests that one of the roles of MCH/MCH-R1 in the skin could be in regulating pigmentation by opposing the actions of the melanocortin system (22). MCH is produced from pro-melanin-concentrating hormone (PMCH). In the human skin the expression of PMCH has been detected in cultured endothelial cells, whereas no signal has been found in keratinocytes, melanocytes and fibroblasts. MCHR1 expression has been demonstrated in melanocytes and melanoma cells (23).

The abnormal expression of CRH-POMC system peptides has been described in a number of skin conditions, such as neoplastic melanocytic naevi, melanoma, basal cell carcinoma, squamous cell carcinoma, inflammatory keloids, and scarring alopecia (11). Our study group has previously demonstrated abnormal gene expression of the melanocortin system in vitiligo skin samples compared with healthy control skin (15). The importance of CRH-POMC system in the pathogenesis of psoriasis is supported by exacerbation and triggering of the disease due to emotional stress and local trauma (24–29). Healing of the psoriatic lesions with temporary hyper- or hypo-pigmentation also implicates the involvement of the CRH-POMC system in the pathogenesis of psoriasis. Until now there have been only a few limited studies that have analysed gene expression levels of CRH-POMC system in psoriasis skin samples.

The aim of the present study was to explore the regulation of the cutaneous stress system in psoriasis by examining the expression variations of genes encoding mediators of the CRH-POMC system and melanogenesis enzymes in lesional and non-lesional psoriasis skin and in the skin of healthy controls.

MATERIALS AND METHODS

The protocols and informed consent forms for this study were approved by the ethics Review Committee on Human Research of the University of Tartu. All of the participants signed a written informed consent.

Patients and healthy controls

The patients and control subjects in the study were Caucasians living in Estonia. Twenty (2 females; 18 males) unrelated patients with plaque-psoriasis, mean age 40.85 years (range 18–64 years) were recruited from the outpatient as well as from the inpatient clinic at the Department of Dermatology, Tartu University Hospital. The mean age of psoriasis onset of the patients was 28.4 years. The Psoriasis Area Severity Index (PASI) score was recorded by the same physician in all cases. The mean PASI score was 25.05 (range 7–69). One patient had PASI less than 7 (mild disease) and all others had PASI over 7 (moderate and severe disease). Ten of the patients had psoriatic nail involvement, 3 had psoriatic arthropathy, and 7 patients had a positive family history of psoriasis. The biopsies were taken from non-sun-exposed areas. Prior to taking the biopsies the patients' group had not received any treatment, including ultraviolet B (UVB)-therapy, local corticosteroids and systemic medication for at least a month. Healthy volunteers free from other dermatoses and from the positive family history of psoriasis, were recruited as a control group ($n = 56$; (18 male, 38 female); Mean age 36.1 years (range 21–70 years). As the female/male ratio differs between study groups it should be clarified that, based on current literature, sex and age do not influence the mRNA expression of studied genes.

Skin samples and TaqMan-quantitative real-time-PCR

Two 4-mm punch biopsies from both involved and non-involved skin were obtained from each patient with psoriasis. One skin biopsy (diameter 4 mm) was obtained from healthy control subjects. All probands had skin phototype II or III, Fitzpatrick classification. Biopsies were instantly snap-frozen and stored at –80ºC until RNA extraction.

For total RNA extraction from skin biopsies the RNeasy Fibrous Tissue Mini Kit (QIAGEN Sciences, Maryland, USA) was used according to the manufacturers' protocol. Biopsies were homogenized by T 10 basic homogenizer (IKA Labortechnik, Staufen, Germany). Extracted RNA was dissolved in RNase-free water and stored at –80ºC until cDNA synthesis. RNA quality was controlled using the NanoDrop ND 1000. For cDNA synthesis 250–500 ng of total RNA, oligoT18 primer and Superscript III reverse transcriptase (Invitrogen Corp., Carlbad, CA, USA) was used according to the manufacturers' protocol.

cDNA was used as a template for TaqMan® QRT-PCR analysis in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Reactions were carried out in 10 µl reaction volumes in 4 replicates. Two primers and a labelled probe were used to detect the mRNA expression level of the reference gene *HPRT-1* (hypoxanthine phosphoribosyl-transferase-1) (HPRT-1 exon 6, 5`-GACTTTGCTTTCCTTGGTCAGG-3`; HPRT-1 exon 7, 5`-AGTCTGGCTTATATCCAACACTTCG-3`; VIC-5`TTTCACCAAGCTTGCGACCTTGA-3`-TAMRA). The expression levels of *POMC*, *MC1R*, *MC2R*, *MC3R*, *MC4R*, *MC5R*, *ASIP*, *TYR*, *T(Y)RP1*, *DCT*, *PMCH*, *CRH*, *CRHR1*,

CRHR2 and *MCHR1* were detected applying TaqMan-QRT-PCR method using TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA). The assay mixes used were: Hs00181770_m1 (ASIP), Hs00157244_m1 (DCT), Hs00165976_m1(TYR), Hs00167051_m1 (TYRP1), Hs00267168_s1 (MC1R), Hs00265039_s1 (MC2R), Hs00252036_s1 (MC3R), Hs00271877_s1 (MC4R), Hs00271882_s1 (MC5R), Hs00174941_m1 (CRH), Hs01062290_m1 (CRHR1), s00266401_m1 (CRHR2), Hs00173595_m1 (PMCH), Hs03044476_m1 (MCHR1), Hs00174947_m1 (POMC).

Statistical analysis

Comparative Ct method (ΔCt value) was used for quantification of mRNA, where the amount of target transcript was normalized according to the level of endogenous reference HPRT-1. Data for studied genes following normal distribution were parametrically tested by unpaired *t*-test, and data not following the normal distribution by Mann–Whitney *U* test using Graphpad Prism 4 software (GraphPad Software, San Diego, CA, USA). For all tests, a *p*-value < 0.05 was considered significant.

RESULTS

Using the quantitative real-time (QRT)-PCR method, mRNA expression levels of 15 genes related to the CRH-POMC system were measured in punch biopsies from lesional and non-lesional skin of patients with psoriasis and from skin of healthy control subjects.

CRH mRNA expression did not reach the detection threshold when 250–500 ng of cDNA was applied to the QRT-PCR reaction. Increased *CRHR1* expression (Fig. 1a) was found in lesional and non-lesional psoriasis skin compared with healthy controls. Specifically, a 3.4-fold increase in *CRHR1* mRNA expression in involved skin compared with healthy subjects ($p < 0.001$) and 3.4-fold increase in *CRHR1* in non-lesional skin compared with healthy controls ($p < 0.01$) was detected.

POMC mRNA expression (Fig. 1b) in lesional skin of psoriasis patients was 2.8-fold higher ($p < 0.001$) compared with healthy control subjects. *POMC* mRNA expression in non-lesional skin of patients with psoriasis was 6.27-fold higher ($p < 0.001$) compared with healthy control subjects.

MC1R expression level was decreased in psoriasis-involved skin compared with uninvolved or healthy control skin. However, these differences were not statistically significant (Fig. 2, graph A). A statistically significant difference was found in *MC2R*, *MC3R* and *MC4R* expression (Fig. 2, graphs B, C, D) between healthy controls and patients with psoriasis. *MC2R* mRNA expression was 8.73-fold higher in non-lesional skin of psoriasis patients compared with healthy subjects ($p < 0.001$). Likewise, the *MC3R* expression level in psoriasis non-lesional skin was 2.41-fold higher ($p < 0.01$) and the *MC4R* expression level was 16.4-fold higher ($p < 0.001$) than in healthy skin. In lesional skin a 10.1-fold increase ($p < 0.001$) in *MC4R* expression was detected compared with healthy control subjects. In psoriasis-affected skin a statistically significant 1.47-fold increase ($p < 0.001$) in the expression of *MC3R* and a 3.3-fold increase in *MC2R* ($p < 0.001$) were detected compared with healthy control skin. *MC5R* mRNA expression levels were decreased in psoriasis lesional and non-lesional skin compared with healthy controls. The differences were not statistically significant (Fig. 2, graph E). The expression of all *MCRs* is shown on a combined figure (Fig. 2). *ASIP* mRNA expression (Fig. 3a) level in lesional skin of patients with psoriasis was 9.8-fold lower ($p < 0.001$) and in psoriasis non-lesional skin 10.1-fold lower ($p < 0.001$) than in the skin of healthy control subjects.

T(Y)RP1 mRNA expression (Fig. 3b) was 2-fold lower ($p < 0.01$) in psoriasis-involved skin and 1.7-fold lower ($p < 0.05$) in psoriasis non-involved skin compared with skin of healthy controls. *TYR* mRNA expression level (Fig. 3c) in lesional skin of psoriasis was 3.7-fold lower ($p < 0.001$) and in psoriasis non-involved skin 2.1-fold lower ($p < 0.01$) compared with healthy control subjects.

Increased *MCHR1* expression (Fig. 3d) in lesional and non-lesional psoriasis skin compared with healthy controls was established. The *MCHR1* mRNA expression was 7.3-fold higher in lesional skin of patients with psoriasis ($p < 0.001$) and 8.6-fold higher in psoriasis non-lesional skin ($p < 0.001$) compared with healthy control subjects.

No statistically significant differences in *CRHR2*, *DCT* and *PMCH* mRNA expression were detected between any of the study groups.

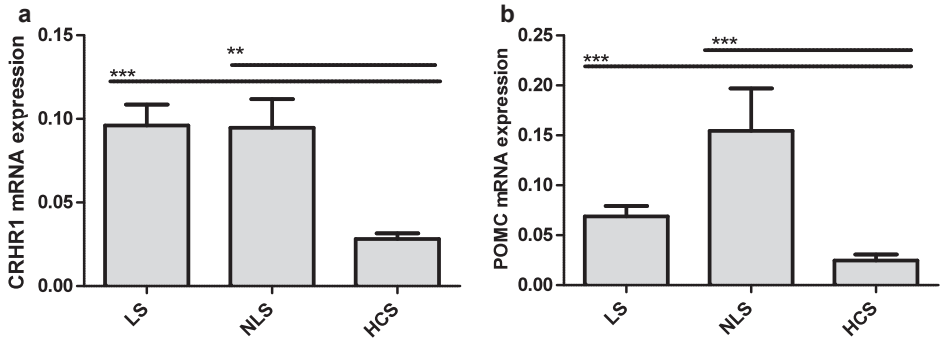

Fig. 1. (a) *CRHR1* mRNA and (b) *POMC* mRNA expression level in psoriasis lesional skin (LS). *CRHR1* mRNA expression level was 3.4-fold higher and in psoriasis non-lesional skin (NLS) 3.4-fold higher compared with healthy control subjects (HCS). *POMC* mRNA expression level was 2.8-fold higher and in psoriasis NLS 6.27-fold higher compared with HCS. Results are displayed as mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$.

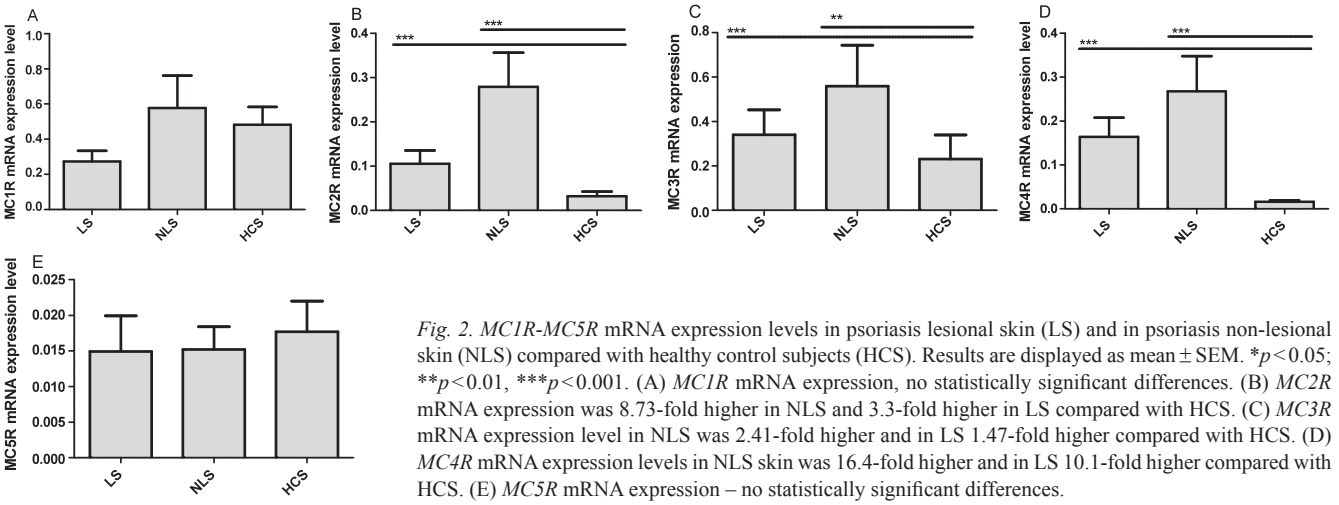

Fig. 2. MC1R-MC5R mRNA expression levels in psoriasis lesional skin (LS) and in psoriasis non-lesional skin (NLS) compared with healthy control subjects (HCS). Results are displayed as mean ± SEM. *$p<0.05$; **$p<0.01$, ***$p<0.001$. (A) *MC1R* mRNA expression, no statistically significant differences. (B) *MC2R* mRNA expression was 8.73-fold higher in NLS and 3.3-fold higher in LS compared with HCS. (C) *MC3R* mRNA expression level in NLS was 2.41-fold higher and in LS 1.47-fold higher compared with HCS. (D) *MC4R* mRNA expression levels in NLS skin was 16.4-fold higher and in LS 10.1-fold higher compared with HCS. (E) *MC5R* mRNA expression – no statistically significant differences.

DISCUSSION

In the present study the expression levels of genes related to CRH-POMC system of human skin were analysed: *POMC*, *MC1R-MC5R*, *ASIP*, *CRH*, *CRHR1*, *CRHR2*, *MCHR1*, *PMCH*; and the melanogenesis enzymes *TYR*, *T(Y)RP1* and *DCT*.

Until now, no expression of *DCT*, *TYR* and *T(Y)RP1* mRNA has been investigated in psoriasis. In our previous studies of vitiligo skin samples we found decreased expression of *DCT*, *TYR* and *T(Y)RP1* mRNA in vitiligo lesional skin (20, 30). In the present study we found statistically significant expressional down-regulation of *TYR* and *T(Y)RP1* mRNA, genes involved in melanin synthesis, in lesional and non-lesional skin of psoriasis patients compared with healthy controls. Even though *DCT* was down-regulated in lesional skin of psoriasis compared with non-lesional skin and healthy controls, no statistically significant differences in *DCT* mRNA expression were detected. This down-regulation of melanogenesis enzymes could explain why psoriatic lesions heal with temporary hypopigmentation.

In our study we found up-regulation of *POMC* mRNA in psoriasis lesional and non-lesional skin compared with healthy controls. In previous studies using immunohistochemical and *in situ* reverse-transcription (RT)-PCR techniques, Kono et al. (31) demonstrated co-expression of *CRH* and *POMC* mRNAs in the epidermis and pilosebaceous units of the human skin. Immunoreactivities and expression of *CRH* and *POMC* mRNAs were strong in inflammatory lesions, such as psoriatic and parapsoriatic lesions and other skin diseases. Kim et al. (32) showed in their study that the expression of POMC peptides ACTH and a-MSH was clearly stimulated in a subset of psoriasis patients compared with controls, but, on the whole, lacked

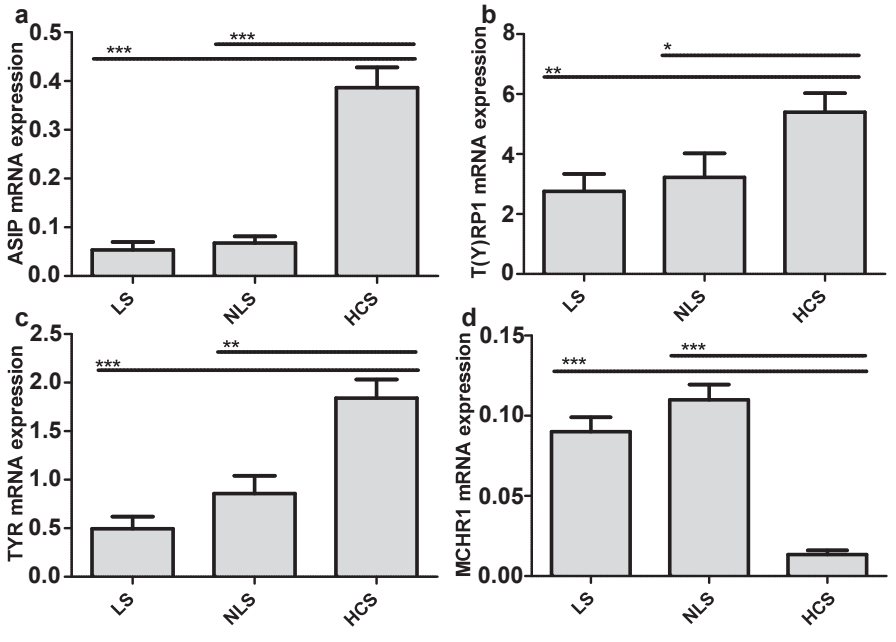

Fig. 3. (a) *ASIP* mRNA, (b) *T(Y)RP1* mRNA, (c) *TYR* mRNA and (d) *MCHR1* mRNA expression level in psoriasis lesional skin (LS). *ASIP* mRNA expression level was 9.8-fold lower, and 10.1-fold lower in psoriasis non-lesional skin (NLS) compared with healthy control subjects (HCS). *T(Y)RP1* mRNA expression level was 2-fold lower, and 1.7-fold lower in psoriasis NLS compared with HCS. *TYR* mRNA expression level was 3.7-fold lower, and 2.1-fold lower in psoriasis NLS compared with HCS. *MCHR1* mRNA expression level was 7.3-fold higher, and 8.6-fold higher in psoriasis NLS compared with HCS. Results are displayed as mean ± SEM. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

statistical significance. It has been shown in previous studies that POMC-derived peptide α-MSH has a direct immunoregulatory and anti-inflammatory role (33), it antagonizes the effects of proinflammatory cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, and tumour-necrosis-factor-α (TNF-α) or endotoxins, and down-regulates the production of proinflammatory cytokines and accessory molecules on antigen-presenting cells, while production of suppressor factors, such as IL-10, is up-regulated by α-MSH (32). High expression of the proinflammatory cytokine IL-6 has been found in psoriatic lesional skin (34). Up-regulation of *POMC* mRNA in lesional and non-lesional psoriatic skin, established in the present study, may attempt to inhibit the action of proinflammatory factors and thereby make an effort to suppress inflammatory responses on the local level. In addition, increased levels of *POMC* mRNAs could, in some cases, explain healing of the psoriatic lesions with transitory hyperpigmentation.

POMC peptides exert their effects via 5 subtypes of MCR (32). The mRNA expression levels of the MCR MC2R, MC3R and MC4R were increased in lesional and non-lesional skin compared with healthy subjects, the differences were statistically significant. Over-expression of MCR in lesional and non-lesional skin possibly indicates the existence of compensatory system to suppress up-regulation of inflammatory cytokines in psoriatic lesions.

We found statistically significant down-regulation of ASIP mRNA in psoriasis lesional and non-lesional skin compared with skin of control subjects. ASIP inhibits the binding of POMC derivate peptide α-MSH to MC1R (21). As up-regulation of POMC was found in lesional and non-lesional psoriatic skin, the down-regulation of ASIP supports our findings that the pigmentation of psoriasis is related to increased levels of POMC in the skin. This finding makes our results biologically meaningful and feasible.

Tagen et al. (35) found CRH-R1 mRNA expression in psoriasis skin to be lower than that in normal controls. There was no statistically significant difference in CRH-R2 mRNA expression among the study groups. Using immunohistochemistry Zhou et al. (36) found that, in psoriatic skin, CRH/CRH-R1 expression was significantly lower compared with a control group. Contrary to those results we found statistically significant increases in the expression of CRHR1 mRNA in lesional ($p < 0.001$) and non-lesional ($p < 0.01$) psoriatic skin compared with healthy controls. Similarly, increased expression of CRHR1 has been shown in the affected skin of patients with other inflammatory skin diseases, such as contact dermatitis and chronic urticaria (33). The functional role of elevated CRHR1 expression in the skin of patients with psoriasis remains to be identified.

There are similar changes in lesional and non-lesional skin compared with healthy subjects. We think that this is because the CRH-POMC system tries to compensate the inflammation in the lesional skin. The changes could be more marked in non-lesional skin, as the CRH-POMC system fights inflammation more aggressively. The findings indicate that psoriasis involves the whole skin, not only the areas with visible lesions.

In conclusion, the expression of genes of the CRH-POMC system and enzymes of melanogenesis is altered in psoriasis. The up-regulation of POMC, CRHR1 and MCHR1 in the lesional and non-lesional skin of patients with psoriasis supports the importance of the local CRH-POMC system in the pathogenesis of psoriasis. Up-regulation of the MCR in non-lesional and lesional skin and decreased expression of ASIP and enzymes of melanogenesis in the lesional and non-lesional skin probably indicates the existence of a compensatory system to inhibit the production of proinflammatory factors in the skin of patients with psoriasis.

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