

Low vitamin D-modulated calcium-regulating proteins in psoriasis vulgaris plaques: S100A7 overexpression depends on joint involvement

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Abstract. Psoriasis is an inflammatory skin disease with or without joint involvement. In this disease, the thickened epidermis and impaired barrier are associated with altered calcium gradients. Calcium and vitamin D are known to play important roles in keratinocyte differentiation and bone metabolism. Intracellular calcium is regulated by calcium-sensing receptor (CASR), calcium release-activated calcium modulator (ORAI) and stromal interaction molecule (STIM). Other proteins modulated by vitamin D play important roles in calcium regulation e.g., calbindin 1 (CALB1) and transient receptor potential cation channel 6 (TRPV6). In this study, we aimed to investigate the expression of calcium-regulating proteins in the plaques of patients with psoriasis vulgaris with or without joint inflammation. We confirmed low calcium levels, keratinocyte hyperproliferation and an altered epidermal barrier. The CASR, ORAI1, ORAI3, STIM1, CALB1 and TRPV6 mRNA, as well as the sterol 27-hydroxylase (CYP27A1), 25-hydroxyvitamin D₃ 1- α -hydroxylase (CYP27B1) and 1,25-dihydroxyvitamin D₃ 24-hydroxylase (CYP24A1) protein levels were low in the plaques of patients with psoriasis. We demonstrated S100 calcium-binding protein A7 (S100A7) overexpression in the plaques of patients with psoriasis vulgaris with joint inflammation, compared with those without joint involvement. We suggest an altered capacity to regulate the intracellular Ca²⁺ concentration ([Ca²⁺]_i), characterized by a reduced expression of CASR, ORAI1, ORAI3, STIM1, CALB1 and TRPV6 associated with diminished levels of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], which may be associated with an altered balance between keratinocyte proliferation and differ-

entiation in the psoriatic epidermis. Additionally, differences in S100A7 expression depend on the presence of joint involvement.

Introduction

The normal epidermal barrier results from an equilibrated differentiation process, in which proliferative undifferentiated keratinocytes move from the basal to the granular layer, turning to a differentiated state of the cornified envelope (CE) (1). Epidermal differentiation involves the expression switch from basal keratin 5 (KRT5) and KRT14 to suprabasal KRT1 and KRT10 (2,3). In addition, structural proteins, such as involucrin (IVL) and periplakin (PPL) are crosslinked with other proteins and serve as substrates for lipids in the CE (4-6). Psoriasis is a common chronic inflammatory skin disease with or without joint involvement. Psoriatic skin lesions are characterized by a thickened epidermis due to increased keratinocyte proliferation, abnormal differentiation and the infiltration of inflammatory cells into the dermis and epidermis. KRT6 and KRT16, markers of abnormal hyperproliferation, are upregulated in psoriatic lesions, whereas KRT1 and KRT10, markers of terminal differentiation, are downregulated (7). Calcium is a major regulator of keratinocyte differentiation. Alterations in calcium levels from 0.03 to 0.1 mM trigger keratinocyte differentiation *in vitro* (8). In the epidermis, calcium gradients from low levels in the proliferative basal layer to high levels in the differentiated granular layer, have been reported (9). This gradient disappears after barrier disruption (10) or in psoriasis (11).

In vitro data have demonstrated that the extracellular Ca²⁺ concentration ([Ca²⁺]_o) initiates keratinocyte differentiation by increasing the intracellular Ca²⁺ concentration ([Ca²⁺]_i). This process is regulated by proteins of the plasma membrane and endoplasmic reticulum, such as the calcium-sensing receptor (CASR) (12,13) and store-operated Ca²⁺ entry (SOCE) proteins (14). It has recently been demonstrated that the two major SOCE components, stromal interaction molecule (STIM) and calcium release-activated calcium modulator (CRAC or ORAI) (15-17) are involved in keratinocyte proliferation and differentiation (18). Moreover, the epidermis is the major source of the calcium homeostasis regulator, vitamin D. Keratinocytes metabolize vitamin D to the active 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] by several cytochrome P450 enzymes (19). This metabolite regulates epidermal proliferation in the basal layer and

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Abbreviations: HC, healthy controls; PV, psoriasis vulgaris; PVPsA, psoriasis vulgaris with joint inflammation; CE, cornified envelope

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promotes differentiation in the upper layers after binding to the vitamin D receptor (VDR) (20). In addition to CASR (21), other calcium-regulating proteins are modulated by $1,25(\text{OH})_2\text{D}_3$; e.g., the calcium-binding calbindin 1 (CALB1) is involved in early keratinocyte differentiation (22). Additionally, transient receptor potential subfamily V member 6 (TRPV6) is a highly selective Ca^{2+} channel upregulated by $1,25(\text{OH})_2\text{D}_3$ (23) and is involved in $\text{Ca}^{2+}/1,25(\text{OH})_2\text{D}_3$ -induced keratinocyte differentiation (24). On the other hand, antimicrobial peptides found in the epidermis, such as S100 calcium-binding protein A7 (S100A7) (25), which bind calcium and zinc (26), are inducible in keratinocytes by differentiation (27), and are highly overexpressed in psoriasis (28).

Currently, the knowledge about the expression of calcium-regulating proteins in the epidermal plaques of psoriasis vulgaris is limited. In this study, we thus aimed to investigate the gene expression of calcium-regulating proteins in the plaques of patients with psoriasis vulgaris with joint inflammation (PVPsA) and without joint inflammation (PV).

Subjects and methods

Subject characteristics. This study, approved by the Ethics Committee at the Medical Faculty of the Friedrich-Schiller University Jena, Jena, Germany (project 1940-01/07), was conducted according to the principles of the Declaration of Helsinki. Written consent was obtained from all participants prior to enrollment. Patients were diagnosed by dermatologists at the Department of Dermatology of the Jena University Hospital. The presence of joint manifestations was confirmed by power doppler ultrasonography (Esaote, Genoa, Italy) and Rheumascan Xeralite (Mivenion GmbH, Berlin, Germany). Eighteen patients with psoriasis vulgaris and six healthy controls (HC) were included. Seven patients with psoriasis vulgaris had no clinical signs of joint inflammation (PV) and eleven were diagnosed with psoriatic arthritis (PVPsA). The mean age ranges of the patients were as follows: PV group, 45.1 ± 22.7 ; PVPsA group, 51.9 ± 14.9 ; and HC group, 48.7 ± 9.9 . Patients with other types of psoriasis, skin diseases, allergy, autoimmune diseases, any topical or systemic treatment, including vitamin D supplementation or phototherapy 5 months before or at the time of recruitment, were excluded.

Skin biopsies. One centimeter biopsies were obtained from patient lesional and control skin after local anesthesia. Biopsies were divided into two 5-mm pieces: one was preserved frozen in 'RNA Later' solution for quantitative polymerase chain reaction (PCR) assessment, and the other embedded in paraffin for posterior 5 μm thicknesses sectioning and processing with Alizarin Red S staining and immunohistochemistry.

Alizarin Red S staining for calcium deposition. Dewaxed skin section slides were incubated for 2 min with Alizarin Red S solution (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The slides were mounted in 'DPX mounting medium' (Sigma-Aldrich, St. Louis, MO, USA) and evaluated under a Zeiss Axio Imager M1 microscope (Carl Zeiss, Jena, Germany).

Quantitative PCR analysis of skin differentiation markers and calcium-regulating proteins. Total RNA was isolated from the

skin biopsies using an RNeasy Fibrous Tissue kit (Qiagen, Hilden, Germany) and then reverse transcribed into cDNA using the high capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The KRT10, KRT16, IVL, PPL, CASR, ORAI1, ORAI3, STIM1, VDR, CALB1, TRPV6 and S100A7 mRNA levels were quantified using a 7500 TaqMan Real-Time PCR system with human KRT10 (Hs01043114_g1), KRT16 (Hs00955088_g1), IVL (Hs00846307_s1), PPL (Hs00160312_m1), CASR (Hs01047793_m1), ORAI1 (Hs03046013_m1), ORAI3 (Hs00743683_s1), STIM1 (Hs00162394_m1), VDR (Hs00172113_m1), CALB1 (Hs01077197_m1), TRPV6 (Hs01114089_g1), S100A7 (Hs01923188_u1) and GAPDH (endogeneous control) TaqMan Gene Expression assays (all from Applied Biosystems). According to the manufacturer's instructions, 50 ng cDNA/50 μl final volume PCR reaction mix were used under the following conditions: an initial AmpErase Uracil N-glycosylase activation step at 50°C for 2 min followed by 10 min denaturation at 95°C, and 40 cycles as follows: 15 sec at 95°C and 1 min at 60°C. Relative quantification was performed using $\Delta\Delta\text{Ct}$ method, as previously described (29).

Immunohistochemistry of skin biopsies. Dewaxed and rehydrated slides were processed for antigen unmasking. Briefly, the slides were boiled in Tris-EDTA buffer (10 mM Tris Base, 1 mM EDTA, 0.05% Tween-20, pH 9.0) for 20 min and then cooled with tap water. The slides were permeabilized for 15 min and blocked for 30 min at room temperature. The samples were incubated overnight at 4°C with the diluted 1:200 primary antibodies [anti-human KRT10 (sc-51581), KRT16 (sc-53255), IVL (sc-15225), PPL (sc-16754), CASR (sc-32182), ORAI1 (sc-377281), ORAI3 (sc-292104), STIM1 (sc-68897), VDR (sc-1009), CALB1 (sc-365360), TRPV6 (sc-28763), S100A7 (sc-67047), sterol 27-hydroxylase (CYP27A1) (sc-390974), 25-hydroxyvitamin D₃ 1- α -hydroxylase (CYP27B1) (sc-49643) and 1,25-dihydroxyvitamin D₃ 24-hydroxylase (CYP24A1) (sc-66851); all from Santa Cruz Biotechnology, Inc.]. After washing, the slides were incubated 2 h at room temperature with the 1:400 diluted secondary antibodies [Alexa Fluor 488 (A-21441)-, 594 (A-11058)- and 647 (A-31571)-conjugated; Molecular Probes, Eugene, OR, USA]. Additionally, the slides were incubated for 5 min with 2 $\mu\text{g}/\text{ml}$ DAPI (Sigma-Aldrich) and observed under a Zeiss Axio Imager M1 microscope (Carl Zeiss).

Statistical analyses. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). Differences between groups were analyzed by the Mann-Whitney or t-test. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Low calcium levels in plaques of patients with psoriasis. The normal epidermis is characterized by a calcium gradient which ranges from low levels in the basal layer to high levels in the spinous and granular layers (30). In this study, we performed Alizarin Red S staining, which is usually used to identify

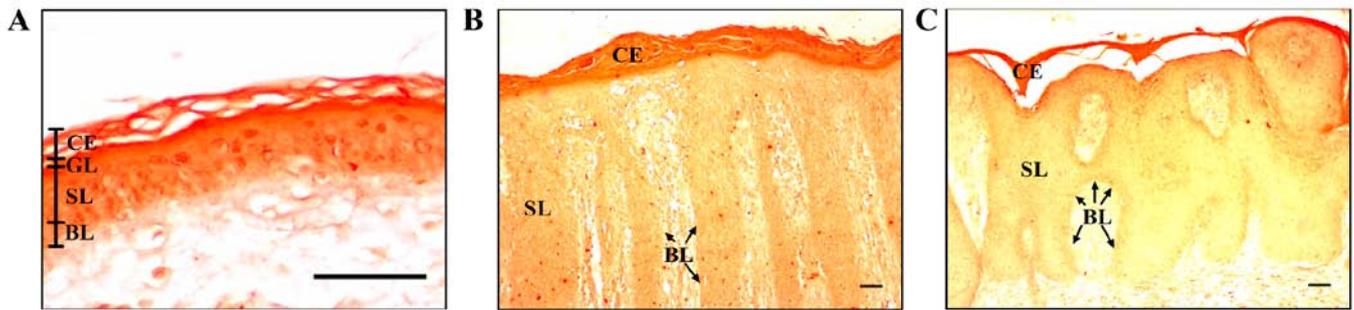


Figure 1. Calcium staining in the plaques of patients with psoriasis vulgaris with or without joint inflammation, and the control epidermis. Light microscopic images of skin sections from (A) healthy controls (HC), and in the plaques of psoriasis vulgaris patients (B) without joint inflammation (PV) and (C) with joint inflammation (PVPsA) were incubated with Alizarin Red S solution. BL, basal layer; SL, spinous layer; GL, granular layer; CE, cornified envelope. Scale bar, 100 μ m.

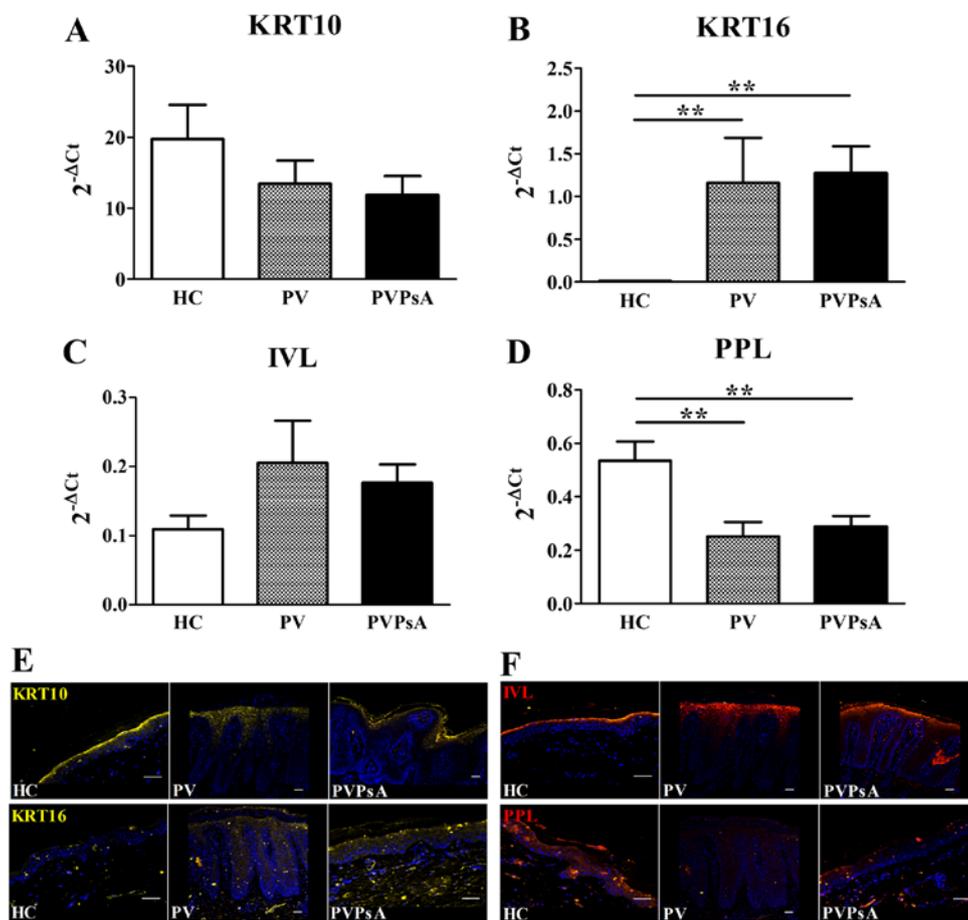


Figure 2. Keratinocyte hyperproliferation and epidermal differentiation markers in patients with psoriasis vulgaris with or without joint inflammation and healthy controls. Quantitative PCR analysis of skin biopsies from healthy controls (HC), and patients with psoriasis vulgaris without joint inflammation (PV) and with joint inflammation (PVPsA). Columns represent the means \pm SEM of $2^{-\Delta C_t}$ (x-axes) of (A) keratin 10 (KRT10), (B) KRT16, (C) involucrin (IVL) and (D) periplakin (PPL). HC, n=6; PV, n=7; PVPsA, n=11. ** $P < 0.01$. (E and F) Microscopic images representative of immunofluorescence staining of (E) KRT10 and KRT16, and (F) IVL and PPL, in 5- μ m-thick skin sections from the HC, PV and PVPsA groups. Scale bar, 100 μ m.

calcium in bone cells and tissue sections, to detect calcium in the skin biopsies. This dye forms an orange-red complex with calcium. We observed lower levels of calcium in the epidermis from the biopsies of patients with psoriasis, as evidenced by the light orange staining in the basal and spinous layers, compared with the dark orange observed in the same layers in the control epidermis (Fig. 1). There were no differences in the staining of

the epidermis of the patients with psoriasis vulgaris with or without joint inflammation. Our results confirmed the already reported altered calcium gradient in the epidermis of patients with psoriasis (11).

Keratinocyte hyperproliferation and altered epidermal differentiation in patients with psoriasis. Subsequently, we

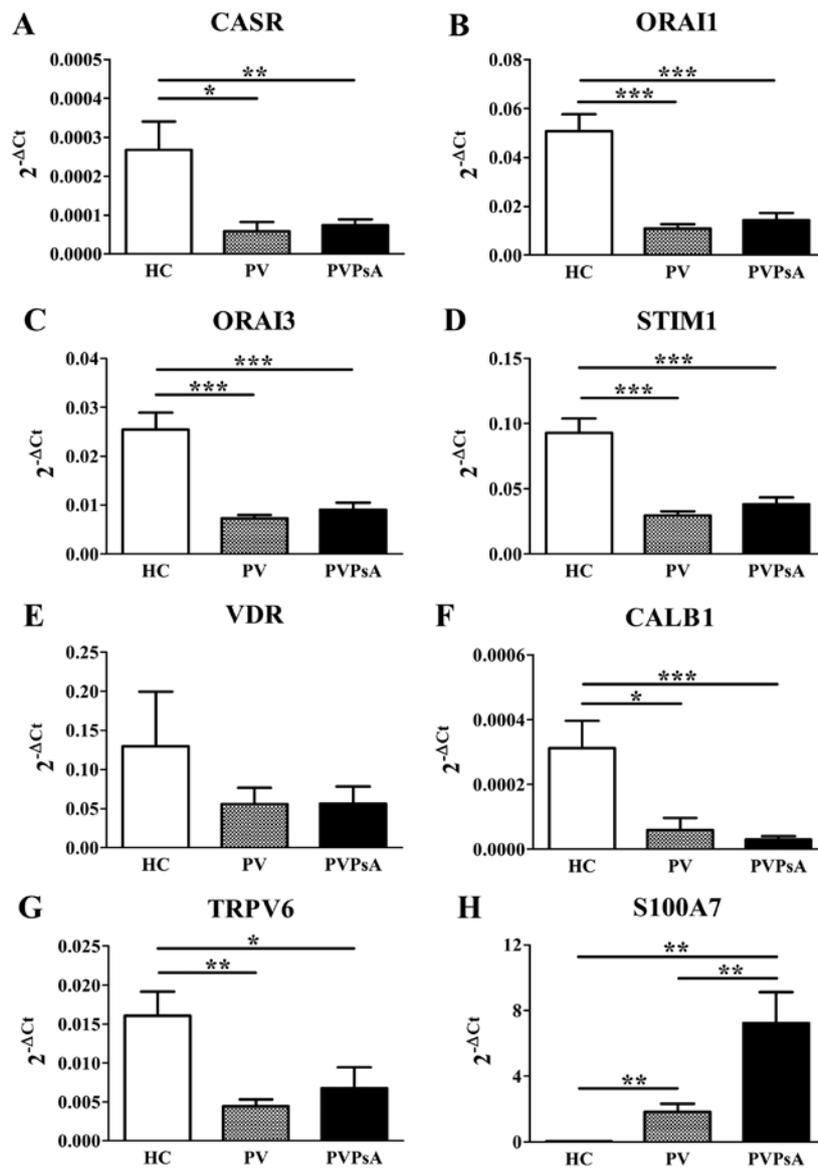


Figure 3. Gene expression of the calcium-modulating proteins, vitamin D receptor (VDR) and S100 calcium-binding protein A7 (S100A7) in plaques of patients with psoriasis vulgaris with or without joint inflammation, and the control epidermis. Quantitative PCR analysis of skin biopsies from healthy controls (HC), and patients with psoriasis vulgaris without joint inflammation (PV) and with joint inflammation (PVPsA). Columns represent the means \pm SEM of $2^{-\Delta Ct}$ (x-axes) of (A) calcium-sensing receptor (CASR), (B) calcium release-activated channel modulator 1 (ORAI1), (C) ORAI3, (D) stromal interaction molecule 1 (STIM1), (E) VDR, (F) calbindin 1 (CALB1), (G) transient receptor potential cation channel 6 (TRPV6) and (H) S100A7. HC, n=6; PV, n=7; PVPsA, n=11. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

assessed the keratinocyte differentiation state by measuring the mRNA expression of keratinocyte differentiation markers in the epidermis from biopsies of patients with PV and PVPsA, as well as in the controls. Our results revealed that the mRNA levels of KRT10, one of the first keratins expressed by keratinocyte differentiation during cornification (31), displayed no statistically significant differences between the PV, PVPsA and control groups ($P>0.05$; Fig. 2A). By contrast, KRT16, a marker for keratinocyte hyperproliferation (7), exhibited highly increased mRNA levels in the plaques of patients with psoriasis compared with the controls (98-fold, $P=0.004$, and 107-fold, $P=0.001$, respectively; Fig. 2B). The early and late keratinocyte differentiation markers, IVL and PPL, respectively, play important roles in the crosslinking of CE proteins (32,33). We did not find any statistically significant differences in the mRNA levels of IVL between the PV, PVPsA and control groups ($P>0.05$;

Fig. 2C). However, the PPL mRNA levels were lower in the plaques of patients with PV and PVPsA compared with the controls (2.1-fold, $P=0.009$, and 1.9-fold, $P=0.006$, respectively; Fig. 2D). At the protein level, we observed a similar expression pattern of KRT10 in the upper spinous layer in the biopsy specimens of patients in the PV, PVPsA and control groups. By contrast, KRT16 was expressed mainly in the spinous layer in the plaques of patients with PV and PVPsA, and was almost absent in the control epidermis (Fig. 2E). Furthermore, IVL was expressed in a similar pattern in the upper spinous layer in biopsy specimens of PV, PVPsA and controls, while PPL was expressed in the spinous and granular layers of the control epidermis, and was almost absent in the plaques of patients with PV and PVPsA (Fig. 2F). These results confirmed altered keratinocyte differentiation in the plaques of patients with psoriasis vulgaris.

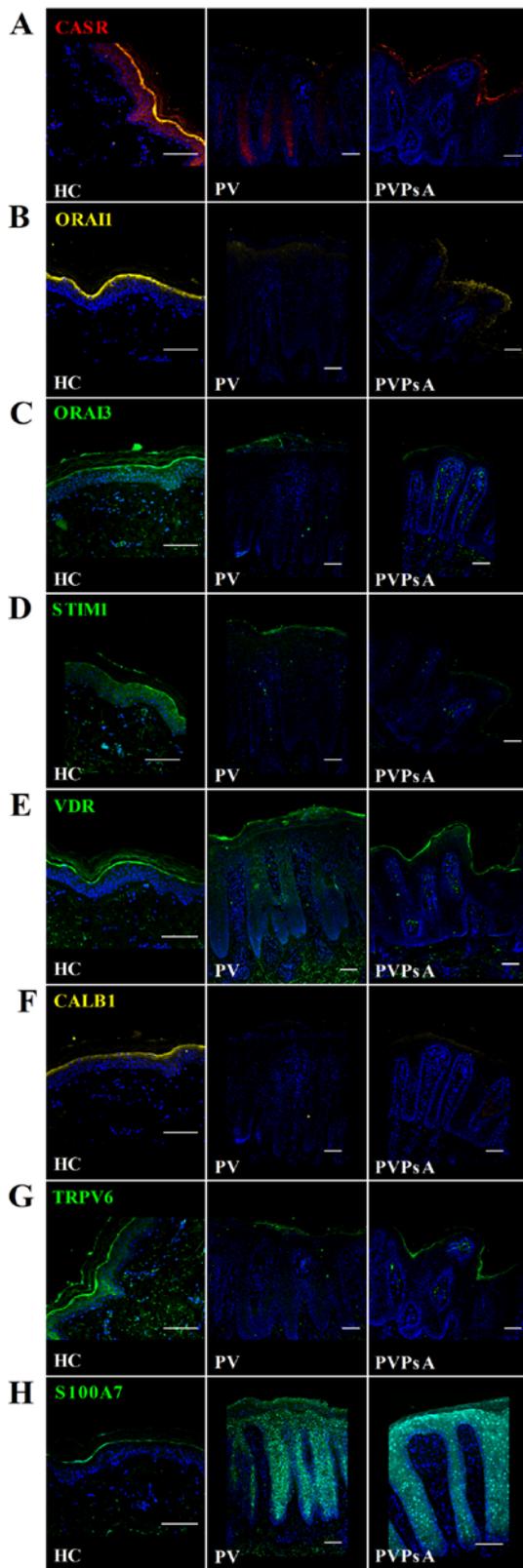


Figure 4. Protein levels of the calcium-modulating proteins, vitamin D receptor (VDR) and S100 calcium-binding protein A7 (S100A7) in plaques of patients with psoriasis vulgaris with or without joint inflammation, and control epidermis. Immunofluorescent microscopic images of skin sections from healthy controls (HC), and patients with psoriasis vulgaris without joint inflammation (PV) and with joint inflammation (PVPsA). Images represent staining of (A) calcium-sensing receptor (CASR), (B) calcium release-activated channel modulator 1 (ORAI1), (C) ORAI3, (D) stromal interaction molecule 1 (STIM1), (E) VDR, (F) calbindin 1 (CALB1), (G) transient receptor potential cation channel 6 (TRPV6) and (H) S100A7. Scale bar, 100 μ m.

Low expression of calcium-regulating proteins and S100A7 overexpression differences in the plaques of patients with psoriasis. $[Ca^{2+}]_o$ plays a critical role in keratinocyte differentiation (34) and $[Ca^{2+}]_i$ is regulated by proteins of the plasma membrane and endoplasmic reticulum (14). Thus, we then evaluated the gene expression of calcium-regulating proteins in the plaques of patients with PV and PVPsA. Our results revealed lower mRNA levels of CASR (4.6-fold, $P=0.012$; 3.6-fold, $P=0.004$, respectively), ORAI1 (4.7-fold, $P<0.0001$; 3.6-fold, $P<0.0001$, respectively), ORAI3 (3.5-fold, $P<0.0001$; 2.8-fold, $P<0.0001$, respectively) and STIM1 (3.1-fold, $P<0.0001$; 2.5-fold, $P<0.0001$, respectively) in the plaques of patients with PV and PVPsA compared with the control epidermis (Fig. 3A-D, respectively).

In addition, $1,25(OH)_2D_3$ is involved in the regulation of keratinocyte differentiation by calcium through VDR (35). Our results revealed no statistically significant differences in the VDR mRNA levels (Fig. 3E). CALB1 is regulated by $1,25(OH)_2D_3$ and is involved in intracellular Ca^{2+} translocation (36). Our results revealed lower CALB1 mRNA levels in the plaques of patients with PV and PVPsA compared with the control epidermis (5.3-fold, $P=0.014$; 10.3-fold, $P<0.0001$, respectively; Fig. 3F). In addition, TRPV6 is another protein regulated by $1,25(OH)_2D_3$ and is a highly selective Ca^{2+} channel (23) involved in keratinocyte differentiation (24). Again, our results revealed lower TRPV6 mRNA levels in the plaques of patients with psoriasis compared with the control epidermis (3.6-fold, $P=0.003$; 2.4-fold, $P=0.014$, respectively; Fig. 3G). By contrast, S100A7, another calcium-binding protein, inducible in keratinocytes by differentiation (27) and involved in the epidermal barrier formation (26), is highly overexpressed in psoriasis (28). Our results confirmed those of published studies by showing a much higher S100A7 gene expression in the plaques of patients with PV and PVPsA compared with the controls (61-fold, $P=0.003$, and 240-fold, $P=0.003$, respectively). Surprisingly, the S100A7 mRNA levels were even greater in the plaques of patients also suffering from joint inflammation (PVPsA) compared those of patients without joint inflammation (PV) (3.9-fold, $P<0.01$; Fig. 3H).

Immunohistochemical staining also revealed that the protein levels of CASR, ORAI1, ORAI3 and STIM were lower in the plaques of patients with PV and PVPsA compared with the control epidermis (Fig. 4A-D). Principally, no differences were observed in the VDR expression pattern in the plaques of patients with psoriasis patients and the control epidermis (Fig. 4E). CALB1 and TRPV6 also displayed a lower protein expression in the plaques of patients with psoriasis patients compared with the control epidermis; e.g., CALB1 localized mainly in the granular layer of control epidermis and was almost absent in psoriatic plaques (Fig. 4F); and TRPV6 was expressed in the spinous and granular layers of the control epidermis and in very low levels in the plaques of patients with PV and PVPsA (Fig. 4G). By contrast, S100A7 expression was higher in the psoriatic plaques compared with the control epidermis. S100A7 localized in the whole epidermis of patients with PV and PVPsA compared with the granular layer of the control epidermis (Fig. 4H).

These results suggest an altered keratinocyte response to $[Ca^{2+}]_o$ and the regulation of $[Ca^{2+}]_i$ in the epidermis of patients with psoriasis. Moreover, the data with S100A7 indicated a

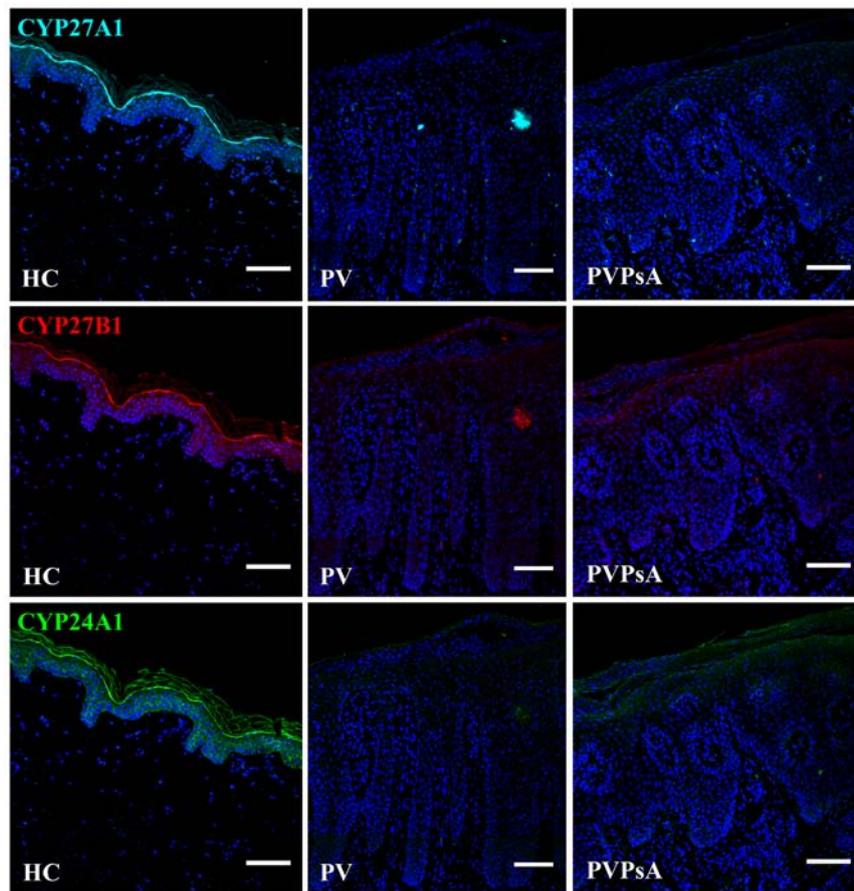


Figure 5. Protein levels of cytochrome P450 enzymes involved in 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] metabolism in plaques of patients with psoriasis vulgaris with or without joint inflammation, and control epidermis. Immunofluorescent microscopic images of skin sections from healthy controls (HC), and patients with psoriasis vulgaris without (PV) and with joint inflammation (PVPsA). Images represent staining of vitamin D₃-25-hydroxylase (CYP27A1), 25-OH-D₃-1 α -hydroxylase (CYP27B1), and 25-OH-D₃-24-hydroxylase (CYP24A1). Scale bar, 100 μ m.

dependence on comorbidity revealed by the mRNA and protein expression differences in the skin of patients with psoriasis vulgaris with or without joint inflammation.

Low protein levels of CYP27A1, CYP27B1 and CYP24A1 in the plaques of patients with psoriasis. The vitamin D active form 1,25(OH)₂D₃ is synthesized by cytochrome P450 enzymes. First, vitamin D₃ is hydroxylated to 25(OH)D₃ by CYP27A1 in the liver (37), then CYP27B1 converts 25(OH)D₃ to 1,25(OH)₂D₃ in the kidneys (38), and CYP24A1 can hydroxylate 1,25(OH)₂D₃, as well as 25(OH)D₃, generating metabolically inactive products (39). Additionally, keratinocytes contain these enzymes (12,40). As these enzymes can be considered markers of vitamin D metabolism, and the expression of the calcium-regulating proteins examined in this study is modulated by 1,25(OH)₂D₃, we then examined their protein levels in the plaques of patients with PV and PVPsA. Our results revealed lower protein levels of CYP27A1, CYP27B1 and CYP24A1 in the plaques of patients with PV and PVPsA compared with the control epidermis (Fig. 5).

Discussion

The beneficial effects of vitamin D induced by exposure to sunlight in the treatment of psoriasis vulgaris have been known for decades. Moreover, the topical application of vitamin D

analogs has been used successfully as the first-line treatment for psoriasis vulgaris (41). In this study, we analyzed vitamin D-dependent, as well as calcium-regulating proteins in the plaques of patients with psoriasis vulgaris. The data presented, schematically summarized in Table I, show an altered expression of differentiation markers in the plaques of patients with psoriasis vulgaris. Although no differences in VDR expression were found, the expression of the calcium-regulating proteins, CASR, ORAI1, ORAI3, STIM1, CALB1 and TRPV6 was reduced, and by contrast, S100A7 was over-expressed in the plaques of patients with psoriasis vulgaris. In addition, the protein levels of CYP27A1, CYP27B1 and CYP24A1 were reduced in the plaques of these patients. Despite the limitation of the use of whole skin gene expression, the results from immunohistochemical analysis confirmed the epidermal expression of these proteins.

Calcium and vitamin D play important roles in keratinocyte differentiation (42,43). In the normal epidermis, calcium gradients have been reported (9). An increase in [Ca²⁺]_o results in the expression of early differentiation markers (44). Low calcium levels confirmed in the plaques of patients with psoriatic correspond to the high expression of the hyperproliferation marker, KRT16. However, no differences in KRT10 expression, a marker of keratinocyte differentiation, were observed between patients with psoriasis and the controls. The integrity of the epidermal barrier is crucial for the main-

Table I. Expression overview of proteins involved in keratinocyte proliferation and differentiation, vitamin D-modulated calcium regulators and metabolic enzymes in plaques of patients with psoriasis vulgaris with or without joint inflammation compared with control epidermis.

Protein	↓	↔	↑
Keratinocyte proliferation and differentiation	PPL	KRT10 IVL	KRT16
Calcium regulation and vitamin D	CASR ORAI1 ORAI3 STIM1 CALB1 TRPV6		S100A7
Vitamin D	CYP27A1 CYP27B1 CYP24A1	VDR	

KRT, keratin; IVL, involucrin; PPL, periplakin; CASR, calcium-sensing receptor; ORAI1, calcium release-activated channel modulator 1; ORAI3, calcium release-activated channel modulator 3; STIM1, stromal interaction molecule 1; VDR, vitamin D receptor; CALB1, calbindin 1; TRPV6, transient receptor potential cation channel 6; S100A7, S100 calcium-binding protein A7; CYP27A1, vitamin D₃-25-hydroxylase; CYP27B1, 25-OH-D₃-1 α -hydroxylase; CYP24A1, 25-OH-D₃-24-hydroxylase. Arrows indicate lower (\downarrow), no change (\leftrightarrow) or higher (\uparrow) protein expression in plaques of patients with psoriasis compared with control epidermis.

tenance of the epidermal calcium gradient (45). According to de Koning *et al* (46) our results demonstrated IVL expressed in the granular layer of control epidermis, but extended into the spinous layer in psoriatic plaques, suggesting a disrupted barrier. In addition, we observed reduced PPL levels in plaques of patients with psoriasis corresponding with an impaired epidermal barrier observed in PPL-deficient mice (47).

Intracellular calcium is regulated by an increase in Ca²⁺ influx through CASR and Ca²⁺ release from intracellular stores, followed by Ca²⁺ re-uptake through SOCE proteins (14,48). CASR is required for normal keratinocyte differentiation (49). The overexpression of CASR accelerates epidermal differentiation, hair follicle formation and permeability (50). while its inactivation or deletion inhibits calcium-induced keratinocyte differentiation by reducing Ca²⁺ intracellular stores, and disrupts epidermal Ca²⁺ gradient and permeability (48,51,52,53). Our results revealed a low CASR expression in the plaques of patients with psoriasis vulgaris, suggesting an altered capacity to regulate [Ca²⁺]_i influx. ORAI and STIM form clusters and co-localize with each other to enable Ca²⁺ influx and release from intracellular stores (54). The knockdown or inhibition of ORAI1 and/or STIM1 alters Ca²⁺ storage and decreases the differentiation and migration of undifferentiated keratinocytes (18). ORAI3 forms heteromultimeric channel complexes with ORAI1 and STIM1, and mutated ORAI1 is sufficient to

exert a negative effect on the other CRAC members (55). In this study, we observed a low expression of ORAI1, ORAI3 and STIM1 in the plaques of patients with psoriasis, suggesting that keratinocytes in these patients have an altered capacity to regulate [Ca²⁺]_i levels.

1,25(OH)₂D₃ increases keratinocyte differentiation by increasing [Ca²⁺]_i levels (35). The loss of VDR or the loss of the capacity to produce 1,25(OH)₂D₃ disrupts epidermal differentiation, resulting in keratinocyte hyperproliferation (56,57). In this study, we did not observe any differences in the expression of VDR. However, the levels of CASR, CALB1 and TRPV6 vitamin D-regulated proteins (58,59), essential in Ca²⁺/1,25(OH)₂D₃-induced differentiation of human keratinocytes (22,24), were reduced in the plaques of patients with psoriasis vulgaris. Other TRP family channels have been shown to be involved in keratinocyte differentiation (60) and complexes with ORAI and STIM have been implicated in the regulation of [Ca²⁺]_i (61). However, only TRPC subfamily members have been investigated in altered Ca²⁺ influx in psoriatic keratinocytes in response to high [Ca²⁺]_o (62).

The binding from 1,25(OH)₂D₃ to VDR and heterodimerization with retinoid X receptors affects the expression of genes that have vitamin D responsive elements in their promoters (63). The expression of genes such as CASR, CALB1, TRPV6 and STIM1 is regulated by 1,25(OH)₂D₃. In addition, 1,25(OH)₂D₃ is a potent regulator of the NF- κ B transcription factor (64), which controls ORAI1 and STIM1 expression (65), and modulates SOCE (66). In accordance with the study by Ala-Houhala *et al* (67), our results revealed lower protein levels of CYP27A1, CYP27B1 and additionally of CYP24A1 in the plaques of patients with psoriasis compared with the control epidermis. The expression of CYP27A1 and CYP27B1 is downregulated by 1,25(OH)₂D₃ (12,68), and the expression of CYP24A1 is induced by 1,25(OH)₂D₃ (69,70), suggesting that low levels of 1,25(OH)₂D₃ are possibly associated with the low calcium-regulating protein levels observed. However, upstream alterations in vitamin D metabolism, e.g., cholesterol metabolism cannot be ruled out. In addition to vitamin D₃, CYP27A1 can hydroxylate cholesterol (71). Elevated cholesterol levels in psoriatic lesioned skin is essential for IL-17A signaling and results in the suppression of genes of cholesterol and fatty acid biosynthesis (72).

1,25(OH)₂D₃ has been shown to exert anti-proliferative effects on keratinocytes (73). Moreover, 1,25(OH)₂D₃ and analogs reduce S100A7 levels in the reconstituted human epidermis stimulated by IL-22 (74), in IL-17-stimulated keratinocytes and in skin of patients with psoriasis (75). Apart from its chemotactic and immunomodulatory functions (76), S100A7, a calcium-binding protein, crosslinks with CE proteins during the terminal stages of keratinocyte differentiation mediated by calcium (77), and is upregulated after epidermal barrier disruption (78) and in psoriatic plaques (28,79). Our results confirmed S100A7 overexpression in the plaques of patients with psoriasis vulgaris, and provide interesting evidence of a higher S100A7 expression in the plaques of patients with PVPsA compared with PV. Bone homeostasis depends on a balance between osteoclasts and osteoblasts. Disordered circulating mediators of bone remodelling (80), and an increased number of circulating osteoclast precursors have been reported in patients with psoriatic arthritis (81). Serum levels of S100A7 are increased in

patients with psoriasis (28). S100A7 has been shown to enhance osteoclast formation *in vitro* (82). Moreover, a S100A7 single nucleotide polymorphism has been shown to be associated with psoriatic arthritis (83).

In conclusion, the altered balance between keratinocyte proliferation and differentiation, together with the altered epidermal barrier observed in psoriatic plaques may be associated with an altered capacity to respond to $[Ca^{2+}]_o$ and to regulate $[Ca^{2+}]_i$, related with a reduced expression of vitamin D-dependent and calcium-regulating proteins, such as CASR, ORAI1, ORAI3, STIM1, CALB1 and TRPV6, as well as with a decreased 1,25(OH) $_2$ D $_3$ synthesis. However, further studies are required to assess the mechanisms involved. In addition, we demonstrated S100A7 overexpression in the plaques of patients with PVPsA compared with PV, suggesting a dependence on the presence of joint inflammation. These data provide new insight into vitamin D-dependent calcium regulation in psoriasis and also reinforce the importance of vitamin D and light therapy in patients with psoriasis with joint inflammation.

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