Glucocorticoid Receptor Beta and Its Prognostic Value on Treatment Response in Chronic Vulvar Dermatitis

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Abstract: Background: Chronic vulvar dermatitis (CVD) is the most prevalent disease in gynecologic dermatology. The treatment mainly depends on topical glucocorticoids (TGC) but is challenged by insufficient treatment response. On a histological level, the upregulation of the glucocorticoid receptor (GR), an inhibitor of the active glucocorticoid receptor (GR), is discussed as mechanism of glucocorticoid insensitivity. Objectives: To analyze whether the expression of GR protein at baseline in keratinocytes may predict responsiveness to TGC in patients with CVD. Methods: In this retrospective cohort study, clinical and biological data of 25 women with a histological diagnosis of chronic vulvar eczema were analyzed. Randomization was done according to the responsiveness to TGC treatment (responsive vs. nonresponsive). Clinical data and the expression of GR in the immunohistochemical stained biopsies were examined. Results: Fifty-two percent of women with CVD were nonresponsive to TGC. GR was abundantly expressed in the cytoplasma of keratinocytes of the vulvar epithelium, but no difference in the level of expression was found among GC responsive and nonresponsive patients in the semiquantitative (p = 0.376) and quantitative analysis (p = 0.894). Conclusion: GR is highly expressed in keratinocytes of the vulvar epidermis affected by CVD, but GR expression was not increased in patients nonresponsive to TGC compared to responsive patients. Thus, the failure mechanism in nonresponders still remains to be elucidated.

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Abstract

Background

Chronic vulvar dermatitis (CVD) is the most prevalent disease in gynecologic dermatology. The treatment mainly depends on topical glucocorticoids (TGC) but is challenged by insufficient treatment response. On a histological level, the upregulation of the glucocorticoid receptor β (GRβ), an inhibitor of the active glucocorticoid receptor α (GRα), is discussed as mechanism of glucocorticoid insensitivity.

Objectives

To analyze whether the expression of GRβ protein at baseline in keratinocytes may predict responsiveness to TGC in patients with CVD.

Methods

In this retrospective cohort study, clinical and biological data of 25 women with a histological diagnosis of chronic vulvar eczema were analyzed. Randomization was done according to the responsiveness to TGC treatment (responsive vs. nonresponsive). Clinical data and the expression of GRβ in the immunohistochemical stained biopsies were examined.

Results

Fifty-two percent of women with CVD were nonresponsive to TGC. GRβ was abundantly expressed in the cytoplasm of keratinocytes of the vulvar epithelium, but no difference in the level of expression was found among GC responsive and nonresponsive patients in the semiquantitative ($p = 0.376$) and quantitative analysis ($p = 0.894$).

Conclusion
GRβ is highly expressed in keratinocytes of the vulvar epidermis affected by CVD, but GRβ expression was not increased in patients nonresponsive to TGC compared to responsive patients. Thus, the failure mechanism in nonresponders still remains to be elucidated.

**Keywords:** Chronic vulvar dermatitis; Glucocorticoids; Glucocorticoid insensitivity; Glucocorticoid receptor beta; Keratinocytes

**Introduction**

Vulvar dermatitis is the most common disease in specialized vulvar clinics [1]. The most frequent cause of vulvar eczema is atopic eczema followed by irritant and allergic contact eczema [2].

Symptoms of vulvar eczema are pruritus, burning, and dyspareunia [3, 4]. Vulvar eczema often undergoes a chronic course which can lead to a significant impact on the quality of life of the affected women [5, 6]. Diagnosis of vulvar eczema is made according to the patient’s history, clinical examination, and histopathology.

Topical glucocorticoids (TGC) are the first-line therapy for vulvar dermatitis though glucocorticoid (GC) insensitivity is thought to play an important role in treatment failure [7–9]. Glucocorticoids exert their anti-inflammatory and immunosuppressive effect by binding the intracellular-located glucocorticoid receptor (GR), belonging to the nuclear receptors superfamily of ligand-dependent transcription factors [10, 11]. The GR is encoded by a single gene composed as a modular protein consisting of 3 domains: an N-terminal transactivation domain, a central DNA-binding domain, and a C-terminal ligand-binding domain [12]. The 2 most important isoforms are GR alpha (GRα) and beta (GRβ) which arise from alternative splicing differing in their last 9th exon [11, 13].

GRα and β are expressed in most human tissue and cell lines but generally GRβ is expressed at a lower level than GRα [13]. However, GRβ was found to be abundant in keratinocytes and cutaneous lymphocytes in inflammatory dermatoses [14]. Only GRα located unliganded in the cytoplasm is able to bind GC, translocate to the nucleus, dimerize and bind GC-responsive elements and therefore down- or upregulate the expression of glucocorticoid-responsive genes [13]. GRβ is unable to bind GC itself but is discussed as an inhibitor of GRα while its precise function remains unclear [13].

In several inflammatory diseases, for example, asthma, chronic rhinitis, or atopic dermatitis, treatment success is limited due to GC insensitivity. An upregulation of GRβ has been described as related to an insensitivity toward GC treatment; however, contradictory data are available [14–18]. The relevance of the expression of GRβ in patients with eczema who are insensitive to TGC treatment remains unclear. To our best knowledge, the expression of GRβ in vulvar eczema has not yet been studied. The objective of this study was to determine whether the expression of GRβ at baseline in keratinocytes of the vulvar epidermis varies among patients responsive and nonresponsive toward TGC treatment.

**Material and Methods**

**Subjects**

After a review of clinical data and tissue specimens, twenty-five (n = 25) female patients treated at the Department of Gynecology of the University Hospital Zurich, Switzerland, between 2016 and 2018 were included in this study. All patients had a clinical and histologically confirmed diagnosis of chronic vulvar dermatitis (CVD). Biopsies were taken after at least 3 months of TGC pause. Treatment and follow-up consultations up to 12 months regarding responsiveness were documented. The research project was approved and registered by the Swiss Ethics Committee (no. BASEC 2019-00037), and a general informed consent for further data and tissue use was obtained from all patients.

All clinical and histological data were pseudonymized (6-digit number). The observers (V.B. and M.P.K.) who conducted further analyses were blinded.

**Treatment**

Women with diffuse erythema and light symptoms were considered to have mild eczema, receiving a topical corticosteroid ointment with low to moderate potency (class I & II: hydrocortisone acetate 25 mg/g or prednisolone acetate 25 mg/g). Women with prominent skin changes (redness and thickening of the skin, blisters, excoriations, and
fissures) and severe impact on their quality of life (severe itching and pain) were considered to have severe eczema, receiving a potent topical steroid (class III [mometasone-17-furoate, 1 mg/g] or IV [clobetasol-17-propionate 0.5 mg/g]). The patients were instructed to apply the GC ointment twice daily for 2 weeks and then taper down. In case of relapse, the therapy could be resumed for up to 6 months [19].

Follow-up consultations were scheduled first between 2 weeks and 6 months after the first consultation, depending on the severity of symptoms, and ended after 12 months. Participants were divided into 2 groups according to their subjective treatment response: responders (remission of symptoms) and nonresponders (insufficient or absent ease of symptoms after at least 2 weeks of treatment).

**Tissue Sample**

Four-mm punch biopsies were obtained and underwent routine histopathologic procedures at the Dermatopathology Laboratory of the Department of Dermatology, University Hospital Zurich. Hematoxylin-eosin (HE) stains were evaluated by 2 trained dermatopathologists (K.K. and I.K.) experienced in the diagnosis of vulvar pathologies.

The histopathological diagnosis of eczema was established on HE stains based on the presence of the following criteria: epithelial changes (acanthosis, spongiosis, exocytosis of lymphocytes, and parakeratosis) and presence of an inflammatory infiltrate in the lamina propria. Periodic acid-Schiff and elastica stainings were routinely performed to exclude mycosis and lichen sclerosus, respectively.

**Immunohistochemistry**

An indirect immunoperoxidase immunohistochemistry (IHC) assay was performed using Vectastain ABC kit (Vector Laboratories). The primary antibody was a rabbit polyclonal antibody (IgG) against human GRβ, dilution 1:2,000 in IHC buffer (ab3581; Abcam, Cambridge, UK). Nonimmunized rabbit IgG (isotype control) (rabbit IgG, I-1000; Vector Laboratories, Burlingame, CA, USA) was used to perform isotype control, and for negative control, the primary antibody was omitted. Human lung tissue served as a positive control, as recommended by the manufacturer. Controls are presented in **Figure 1**.

![Image Analysis](image)

**Image Analysis**

- **a** HE stain of CVD with pronounced acanthosis and spongiosis, exocytosis of lymphocytes, and mild hyperparakeratosis. Marked inflammatory perivascular infiltrate and pigment incontinence in the lamina propria.
- **b** Alveolar epithelium as positive control tissue immunohistochemically stained with GRβ; mast cell stain also positive for GRβ.
- **c** Isotype control on vulvar tissue with nonimmunized rabbit IgG.
- **d** Negative control on vulvar tissue omitting the primary antibody. HE, hematoxylin-eosin; CVD, chronic vulvar dermatitis.
The images were captured using an RGB color video camera (DFC 295; Leica, Wetzlar, Germany) attached to a light microscope (DMI 6000B; Leica, Wetzlar, Germany), NA 0.3, ×10 magnification. Three images per sample were captured showing representative histopathologic features of eczema. Color density and white balance were standardized for all images. Image analysis of the epidermal component was performed using the IHC Profiler plugin for ImageJ version 1 (NIH; Bethesda, MD, USA) (Java 1.8.9_66). A semiquantitative score was assigned accordingly: high positive, positive, low positive, and negative [20]. The optical density score was calculated out of the score generated via the IHC Profiler for quantitative analysis [21]. The intraclass correlation coefficient was calculated for 5 samples with excellent correlation results (0.975). Therefore, we analyzed 1 image per patient for final calculations. A trained specialist (I.K.) performed a semiquantitative analysis of the perivascular infiltrate (light/moderate/severe) with special interest in the expression of GRβ protein in inflammatory cells.

Statistics

In this retrospective cohort study, statistical analysis was performed with the SPSS software package (23.0, SPSS Inc., Chicago, IL, USA). In the descriptive analysis percentages, mean and standard deviation or median and interquartile distance were calculated for clinical, pharmacological, and immunohistochemical variables as appropriate. Mann-Whitney U test was used to compare values from the treatment response and immunohistochemistry in GC responsive and nonresponsive patients. The level of significance was set at \( p < 0.05 \), \( p < 0.07 \) was set as tendency, 1 sided. Because data concerning the mean and standard deviation of GRβ expression in CVD were not available when this project was begun, no power calculation was possible at the beginning of the study. Previous studies on the GR expression in inflammatory dermatoses or rheumatoid arthritis were done with collectives of 15, 13, and resp. 15 patients [14, 22, 23]. As the number of participants was <30, exact significances were used.

Results

Baseline Characteristics

The baseline characteristics are listed according to responders and nonresponders. Group comparison was made and expressed as \( p \) values. Most women with CVD were premenopausal (77%). Atopic predisposition was very common (11/17 patients: positive prick test and/or elevated serum IgE) (Table 1).

| Demographic data, irritative symptoms, atopic predisposition, allergological testings, and gynecological examination, \( n = 25 \) | Responders \(( n = 12 )\) | Nonresponders \(( n = 13 )\) | \( p \) value |
|---|---|---|---|
| Age (mean) | 38.7 | 37.9 | 0.915 |
| Clinical hormonal status, % \(( n/N )\) | | | |
| Premenopausal | 75 (9/12) | 76.9 (10/13) | |
| Perimenopausal | 8.3 (1/12) | 0 (0/13) | 0.611 |
| Postmenopausal | 16.7 (2/12) | 23.1 (3/13) | |
| Hormonal contraception, % \(( n/N )\) | | | |
| Endometriosis | 8.3 (1/12) | 7.7 (1/13) | 0.769 |
| Hemochromatosis | | 7.7 (1/13) | |
| Irritative symptoms, % \(( n/N )\) | | | |
| Pruritus | 75.0 (9/12) | 84.6 (11/13) | 0.689 |
Histology

A total of 25 vulvar biopsies were included. Twenty-one specimens were taken from the vulvar mucosae (in 18/21 cases of the introitus and in 3/21 cases of the labia minora) and 4 specimens showed keratinized epithelium (in 2/4 of the labia minora and in 2/4 of the labia majora). The histological findings are presented in Table 2.

| Burning sensation (%) | Responders (n = 12) | Nonresponders (n = 13) | p value |
|------------------------|---------------------|------------------------|---------|
| 41.7 (5/12)            | 84.6 (11/13)        | 0.110                  |
| Vulvar pain (%)        | 75 (9/12)           | 61.5 (8/13)            | 1       |
| Dyspareunia (%)        | 66.7 (8/12)         | 69.3 (9/13)            | 0.320   |
| Atopic predisposition (%) | 91.7 (11/12)      | 46.2 (6/13)            | 0.077   |
| Atopic dermatitis (%)  | 25 (3/12)           | 0 (0/13)               | 0.295   |
| Allergic rhinitis/conjunctivitis (%) | 25 (3/12) | 38.5 (5/13) | 0.611 |
| Asthma (%)             | 16.7 (2/12)         | 7.7 (1/13)             | 0.979   |
| Positive family history of atopy (%) | 66.7 (8/12) | 23.1 (3/13) | 0.225 |

Histological findings, n = 25

| Histological findings (%) | Responders (n = 12) | Nonresponders (n = 13) | p value |
|---------------------------|---------------------|------------------------|---------|
| Acanthosis (%)            | 100 (12/12)         | 100 (13/13)            | 1       |
| Spongiosis (%)            | 75 (9/12)           | 38.5 (5/13)            | 0.123   |
| Hyper/parakeratosis (%)   | 83.3 (10/12)        | 76.9 (10/13)           | 0.810   |
| Intraepithelial lymphocytes (%) | 91.7 (11/12) | 100 (13/13) | 0.728 |
| Perivascular infiltrate in the lamina propria (%) | 33.4 (4/12) | 23.1 (3/13) | 0.406 |
| Nickel (%)                | 33.4 (4/12)         | 23.1 (3/13)            | 0.406   |
| Amerchol L-101 (%)        | 25 (3/12)           | –                      |         |
| Glucocorticoids (%)       | 0 (0/12)            | 0 (0/13)               |         |

Gynecological examination of the vulva, % (n/N)

| Condition                | Responders (n = 12) | Nonresponders (n = 13) | p value |
|--------------------------|---------------------|------------------------|---------|
| Vulvar erythema (%)      | 66.7 (8/12)         | 100 (13/13)            | 0.168   |
| Dryness (%)              | 41.7 (5/12)         | 30.8 (4/13)            | 0.650   |
| Edema (%)                | 8.3 (1/12)          | 7.7 (1/13)             | 0.979   |
| Thickening of the skin (%) | 50 (6/12)         | 15.4 (2/13)            | 0.152   |
| Excoriations (%)         | 16.7 (2/12)         | 0 (0/13)               | 0.503   |
| Fissures (%)             | 16.7 (2/12)         | 15.4 (2/13)            | 0.979   |
| Blisters (%)             | 0 (0/12)            | 0 (0/13)               | 0.650   |

Table 2.

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.
Glucocorticoid Treatment

Most patients had a history of previous treatments with TGC (16/25), topical antimycotics (21/25), and emollients (25/25). Treatment responses related to GC potency are shown in Table 3. No significant difference between treatment response and TGC potency class was found in our study group ($p = 0.848$).

| Treatment response | Responders ($n = 12$) | Nonresponders ($n = 13$) | $p$ value |
|--------------------|------------------------|--------------------------|-----------|
| TGC class I        | 6                      | 7                        |           |
| TGC class III/IV   | 6                      | 6                        |           |
| Total, % ($n/N$)   | 48 (12/25)             | 52 (13/25)               | 0.848     |

TGC, topical glucocorticoids.

Expression of GRβ

**Epithelium**

We detected positive cytoplasmic staining with GRβ in a large part of the keratinocytes in all tissue samples (25/25) with a predominant expression in the basal layer in 92.3% (24/25) of the cases. We found a positive nuclear GRβ staining of the keratinocytes (13/25) in half of the samples, restricted to different levels of the epithelium as demonstrated in Table 4. We found no difference in the expression of GRβ among mucosa and keratinized epithelium ($p = 0.322$).

Analysis of the nuclear GRβ expression and semiquantitative and quantitative analysis of the total amount of GRβ expression with IHC Profiler and IHC optical density score in the epithelium comparing responders and nonresponders to TGC treatment

| GRβ expression         | Responders ($n = 12$) | Nonresponders ($n = 13$) | Total     | $p$ value |
|------------------------|------------------------|--------------------------|-----------|-----------|
| Nuclear GRβ staining, $n/N$ (%) |                       |                          |           |           |
| Positive               | 4/12 (33.3)            | 9/13 (69.2)              | 13/25 (52)| 0.251     |
| Upper part of the spinous layer | 1/12 (8.3)            | 4/13 (30.8)              | 5/25 (20) |           |
| Lower part of the spinous layer | 2/12 (16.7)           | 2/13 (15.4)              | 4/25 (16) |           |
| Basal layer            | 1/12 (8.3)             | 3/13 (23.1)              | 4/25 (16) |           |
The total amount of expression of GRβ protein varied among the samples as demonstrated in Figure 2 and Table 4. There were no significant differences in the expression of the total amount (semiquantitative: \( p = 0.376 \) and quantitative: \( p = 0.894 \)) or the positive nuclear staining of GRβ (\( p = 0.251 \)) in the epithelium when comparing responsive patients with those nonresponsive to TGC treatment. However, we found positive nuclear staining more frequently in nonresponders (69.2%) than in responders (33.3%).

|                | Semiquantitative, \( n/N \) (%) | Quantitative |
|----------------|---------------------------------|--------------|
| Negative       | 8/12 (66.7)                     | 4/13 (30.8)  | 12/25 (48)                      |
| Low positive   | 6                               | 5            | 11/25 (44)                      |
| Positive       | 5                               | 4            | 10/25 (40)                      |
| High positive  | 1                               | 3            | 5/25 (20)                       |
| IHC optical density score, mean (SD) | 2.29 (0.62) | 2.40 (0.82) | 0.894                       |

GRβ, glucocorticoid receptor β; IHC, immunohistochemistry; TGC, topical glucocorticoids.

Fig. 2.
**Lamina Propria**

We found no difference in the density of the inflammatory infiltrate among responders and nonresponders as shown in Table 2. Analysis of the inflammatory cells in the lamina propria showed positive GRβ expression in the lymphocytes and mast cells in all tissue samples while eosinophilic or neutrophilic granulocytes did not express GRβ. In addition to the inflammatory cells, we also found positive expression of GRβ in vascular endothelial cells as previously described in literature [14, 24].

**Discussion**

In this retrospective study, we analyzed GC responsiveness in CVD related to the expression of GRβ in keratinocytes of the vulvar epithelium. GC insensitivity is a multifactorial problem challenging the treatment of various inflammatory and autoimmune diseases [10]. TGC treatment led to complete remission of symptoms in only half of the patients (48%), while in 52% the treatment was not or not sustainably successful. One suggested molecular mechanism decreasing the anti-inflammatory effect of GCs is the upregulation of GRβ [10, 25].

We detected abundant GRβ protein expression at the whole cell level in keratinocytes in all the vulvar tissue samples. Skin samples from patients with inflammatory skin disorders showed similar expression of GRβ [14, 26], whereas the protein is little expressed in most of the healthy human tissues and cell types [27, 28].

This is supported by the finding that a dysregulation of the endogenous skin GC production can play a key role in skin inflammatory disorders [29]. Human skin cells express the full cytochrome P450 side-chain cleavage system involved in steroid synthesis [30] as well as key high-level regulators such as corticotropin-releasing hormone, proopiomelanocortin, and ACTH [31][32], which can be stimulated by stressors such as UVB or inflammation [32]. It seems possible that this peripheral endogenous GC production is involved in the regulation of the expression of GRβ.

Modulators of GRβ expression include cytokines [33, 34], microbial antigens [4, 35], and superantigens [15]. Notably, streptococcal and staphylococcal antigens are highly prevalent on the skin [36]. These mechanisms might have contributed to the similarly high expression of GRβ in CVD.

Expression of the GRβ protein was predominantly found in the cytoplasm of keratinocytes, whereas nuclear staining of GRβ was only seen in few cases. This may be explained by the fact that GRβ can interfere with GRα on a nuclear level and affect its mediated gene repression [37].

GRβ staining in the epithelium did not show any significant differences among responders and nonresponders to TGC treatment. While this finding is consistent with research on atopic dermatitis [14], GC-resistant patients in other inflammatory diseases, for example, asthma, were shown to have an increased expression of GRβ [14, 17, 38–41]. The function of GRβ likely depends on the involved cell types and may be modulated by disease-specific factors such as cytokines [42]. Intracrinology plays a role in the sex steroid physiology in women (E2 and testosterone) as appropriate intracellular steroid-inactivating enzymes prevent the release of biologically significant amount of E2 or testosterone into the circulation, thus avoiding inappropriate action in the other tissues [43]. Whether intracrinology applies to GC synthesis and contributes to different responsiveness in CVD has to be further elucidated.

As we focused on the epidermal expression of GRβ, future studies should address the expression of GRβ of the dermal component omitting the vascular endothelia. Laser capture microdissection may be predisposed for such work. After
extraction of the RNA and complementary DNA, a quantitative real-time PCR using primers for GRβ and the housekeeping gene would reveal the amount of steroid receptor expression in this specific layer [44].

The application of TGC is described to alter the GR profile [14]. We can discard this factor as none of the patients received TGC or systemic steroids at least 3 months prior to the enrollment in the present study. We were not able to show a difference between the applied TGC potency and treatment response but may have missed such an effect because of the small number of study participants. It is worth noting that the quantification of treatment response in CVD with a standardized recording, for example, the Dermatology Life Quality Index, is not established. We chose an immunohistochemical GR antibody, which was successfully applied in previous studies. However, the fact that there are different immunohistochemical GR antibodies and quantification methods needs to be considered when comparing the literature. Computerized image analysis was used to avoid bias.

The remarkable expression of GRβ in CVD demonstrated herein predisposes this common disease for future studies analyzing the yet not fully understood function of GRβ [45]. More knowledge of the role of GRβ will contribute to a better understanding of its involvement in GC insensitivity and might result in novel treatment strategies for GC-insensitive patients.

In summary, our study reveals that keratinocytes of the vulvar epithelium of patients with CVD abundantly express GRβ protein regardless of TGC responsiveness or nonresponsiveness. Further studies are needed to elucidate the function of GRβ in CVD and its impact on treatment response. Our results suggest that other aspects than the expression of GRβ have to be taken into consideration for treatment success in CVD.

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Statement of Ethics

A general informed consent for further data and tissue use had previously been obtained from all patients. The research project was approved and registered by the Swiss Ethics Committee (no. BASEC 2019-00037).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Valerie Bernays designed the research study, collected the data, performed the analysis, and wrote the paper. Isabel Kolm contributed essential reagents or tools, analyzed the data, and edited the paper. Cornelia Betschart collected the data and edited the paper. Mariusz Pawel Kowalewski contributed essential reagents and tools and edited the paper. Ioannis Dedes and Daniel Fink edited the paper. Katrin Kerl analyzed the data. All authors have read and approved the final manuscript.

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Query: References 31 was not cited in the text. Please indicate where to place the citation.

Answer: Reference 31 should be in the discussion in the third paragraph after the word ACTH. 31 should be written there instead of reference 32.