

## Original Research Article

# Up-regulation of corticotropin releasing hormone is associated with the downregulation of corticotropin releasing hormone binding protein and up-regulation of IL-33 and IL-8 expression in psoriasis

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### Abstract

**Purpose:** To determine the expression of corticotropin releasing hormone (CRH) in psoriasis and normal skin biopsy samples, and to correlate the expression of CRH with the expression of CRHBP and inflammatory cytokines IL-8 and IL-33.

**Methods:** Psoriasis and normal skin biopsy samples were obtained from three psoriatic and three normal healthy patients. The mRNA expression was examined by quantitative real-time polymerase chain reaction (RT-PCR). Protein expression analysis was carried out by Western blotting and then further validated by immunohistochemistry.

**Results:** The results of the present study revealed that the expression of CRH was highly significant ( $p < 0.0001$ ) in psoriatic skin, compared to normal skin. Increase of CRH in psoriatic samples ranged from 2.7 to 3.5-fold. Expression of CRH was associated with the concomitant downregulation of CRHBP in all the psoriatic skin biopsy samples, with expression of CRHBP 3.0 to 6.2-fold lower in psoriatic skin than in normal skin. Analysis of mRNA expression of IL-8 and IL-33, revealed that expression of both IL-8 and IL-33 was significantly ( $p < 0.0001$ ) upregulated in psoriatic skin samples while the expression of IL-8 and IL-33 was approximately 4.1- and 3.2-fold higher in psoriatic skin than in normal skin. The expression of CRHBP, IL-8 and IL-33 was further confirmed by western blotting and immunohistochemistry results.

**Conclusion:** The results confirm that the expression of CRH is associated with the suppression of CRHBP and upregulation of IL-8 and IL-33.

**Keywords:** Psoriasis, Corticotropin releasing hormone, Inflammatory cytokines, Interleukin, Skin biopsy

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## INTRODUCTION

Psoriasis is one of the chronic inflammatory skin disorders that affect around 3 % of the human population around the globe. However, studies have reported that the incidence of psoriasis may vary with race and geographical locality and the environmental conditions [1,2]. Psoriasis has broad clinical spectra ranging from the

involvement of skin to erythroderma [3]. Psoriasis has been categorized into two different types. Type I psoriasis is genetic, usually arises at or around the age of 16 years in females and 22 years in males. Type II psoriasis is sporadic and generally affects the people around 60 years of age [4]. The development of psoriatic plaques triggers pain and generates a lot of discomfort [5].

Psoriasis is accompanied by prolonged inflammation and it frequently co-occurs with inflammatory arthritis [6]. IL-33 is one of the newly discovered members of inflammatory cytokines [6] and has been reported to trigger IgE-triggered release of IL-8 [7]. It is well established in literature that stress signals such as psoriasis prompts the release of CRH from the hypothalamus paraventricular nucleus (PVN). CRH in turn triggers ACTH release from anterior pituitary [8] which ultimately controls the glucocorticoid discharge from adrenal cortex. Several of the glucocorticoids, which include but are not limited to cortisol, at low doses trigger cutaneous immune and suppress the immune responses at high doses [9]. In the sympathetic nervous system, stress stimuli cause activation of the locus coeruleus (LC) of norepinephrine cells (NE) [10]. The neuropeptides which are products of the sympathetic response substance P (SP), cutaneous nerve growth factor (NGF) and calcitonin gene-related peptide (CGRP), are reported to be pro- and anti-inflammatory depending on the type of the immune [11-13]. Moreover, it has been reported that CRH is tightly bound to a high affinity plasma binding protein (CRHBP) and CRHBP limits the distribution of CRH in the body [14].

In the current study, we examined the expression of CRH in psoriasis biopsy samples and correlated its expression with IL-8, IL-33 and CRHBP. The results revealed that expression of CRH is positively associated with expression of interleukin IL-8, IL-33 and negatively correlated with the expression of CRHBP.

## EXPERIMENTAL

### Collection of samples

Biopsies were taken from normal skin tissues of healthy volunteers as well as untreated psoriasis patients. The proper consent was given by the patients under protocols approved by the Institutional Review Board of Wuhan No.1 Hospital (no. 2016/IEC/S027). For immunohistochemistry analysis the samples were treated with formalin and paraffin embedded. The tissue samples were used for isolation of RNA, quantification protein expression levels of CRH, CRHBP, IL-8 and IL-33 as described previously [15, 16]. The study was carried out after the ethical approval was provided by the ethical committee of Wuhan No.1 Hospital. The whole procedure was carried out according to international guidelines [17].

### RNA isolation, synthesis of cDNA and expression analysis

Total RNA was isolated by RNeasy RNA isolation kit (Qiagen, China Shanghai Co Ltd) as per the manufacturer's protocol. Thereafter, cDNA was synthesized with the help of RevertAid cDNA synthesis kit (Fermentas) as per the guidelines provided by the manufacturer.

To carry out the quantification of the mRNA levels, the cDNA was first diluted at least 20 times and qRT-PCR was carried out thrice in ABI StepOne Real time (Applied biosystems), California, United States) using SYBR Green Master Mix (Fermentas, Massachusetts, United States). The relative quantification method ( $\Delta\Delta$ -CT) was employed to determine quantitative variation between the samples examined.  $\beta$ -Actin was used as positive control.

### Western blotting

The skin tissues were lysed in lysis buffer and protein extracts were collected. Equal protein extracts from each group were run on SDS PAGE and then transferred to a polyvinylidene fluoride membrane. This was followed by blocking with 5 % non-fat milk and incubation at 25 °C for 1h.

Thereafter the membranes were administrated with a specific primary antibody at 4 °C overnight. This was followed by washing in washing buffer and incubation for 1h with appropriate secondary antibody. The protein bands of interest were visualized with an ECL Advanced Western Blot Detection Kit.

### Immunohistochemistry

For immunohistochemistry, monoclonal mouse anti-human antibodies to IL-33 and IL-8 were obtained from Invitrogen, Carlsbad, CA. The binding of the mouse antibody was demonstrated in skin biopsies with the help of biotinylated horse anti-mouse IgG and an avidin-biotin complex used with a peroxidase visualization reaction as per the guidelines provided by the manufacturer.

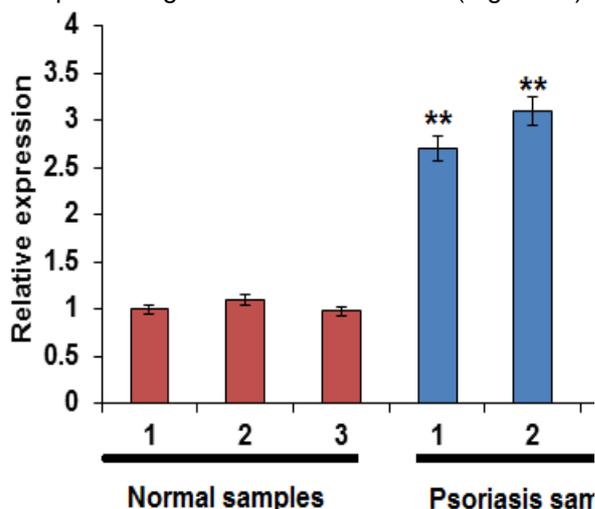
### Statistical analysis

The data is presented as mean of three replicates  $\pm$  SD. GraphPad Prism 7 software was employed to carry out one way ANOVA followed by Tukey's post hoc test. The values were considered significant variously at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$ .

## RESULTS

### Expression of CRH is highly induced in psoriasis

It is well reported that expression of CRH is induced under stress conditions, therefore we evaluated the expression of CRH in healthy and psoriatic skin biopsy samples. The results revealed that expression of CRH was highly upregulated in all the psoriatic biopsy samples as compared to normal skin samples. The increase in the expression of CRH in psoriatic skin samples ranged from 2.7-3.5-fold (Figure 1).

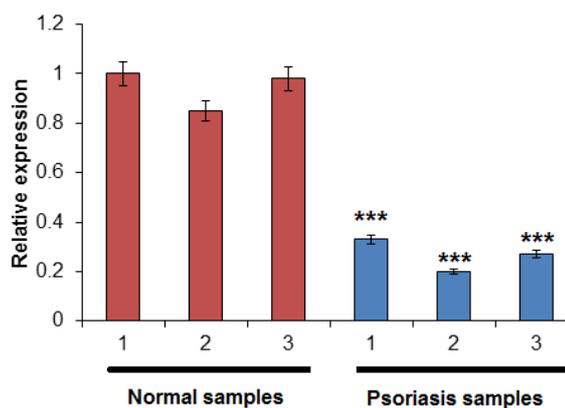


**Figure 1:** Expression of CRH in normal and psoriasis skin biopsy samples determined by semi-quantitative RT-PCR analysis. Experiments were carried out in three biological replicates and presented as mean  $\pm$  SD. The values were considered significant at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$

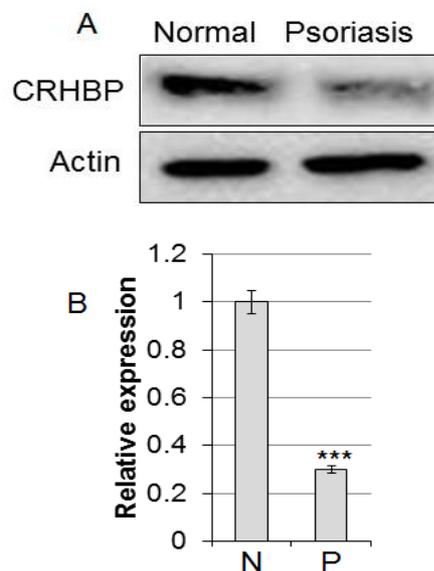
### Expression of CRH is associated with downregulation of CRHBP

Studies have reported that CRH is tightly bound to CRHBP that limits its distribution [18]. Therefore, we evaluated the expression of CRHBP in psoriatic and normal skin biopsy samples by quantitative RT-PCR. The results of the present investigation revealed that the expression of CRHBP was significantly inhibited in the psoriatic samples in comparison to the normal skin samples.

The expression of CRHBP was 3.0 to 6.2-fold lower in the psoriatic skin than in normal skin (Figure 2). To further, confirm this we evaluated the expression of CRHBP in one normal and one psoriatic skin biopsy samples by western blotting. The results of western blotting showed similar trend as that of RT-PCR analysis (Figure 3).



**Figure 2:** Expression of CRHBP in normal and psoriasis skin biopsy samples determined by semi-quantitative RT-PCR analysis. Experiments were carried out in three biological replicates and presented as mean  $\pm$  SD. The values were considered significant at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$

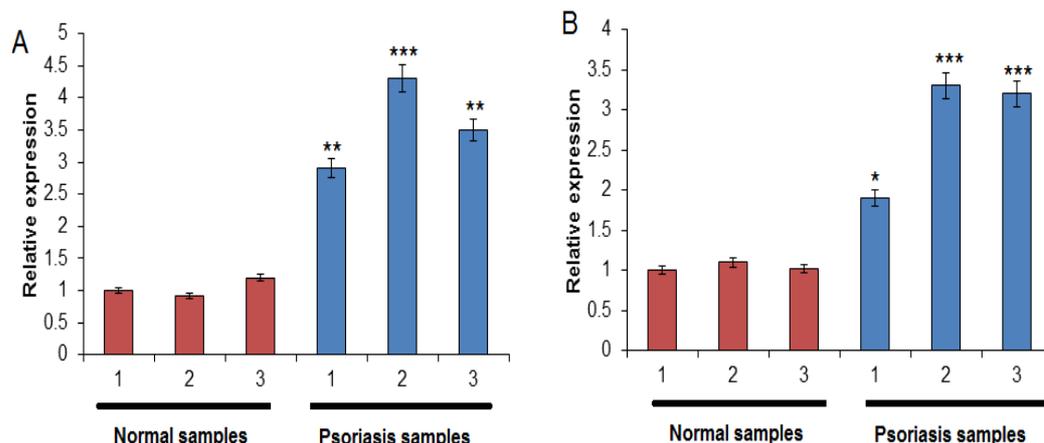


**Figure 3:** Protein expression of CRHBP in normal and psoriasis skin biopsy samples determined by western blot analysis. Experiments were carried out in three biological replicates and presented as mean  $\pm$  SD. The values were considered significant at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$

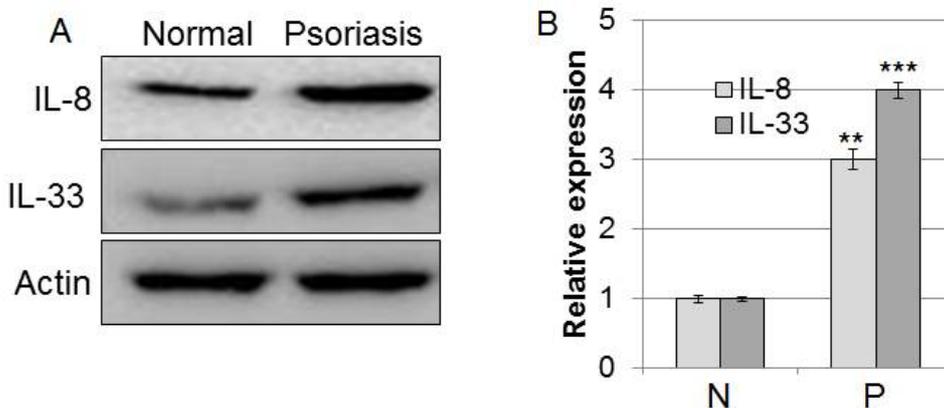
### Expression of CRH is associated with upregulation of IL-8 and IL-33

Psoriasis is one of the chronic inflammatory skin disorders and is always associated with prolonged inflammation [19]. In the current study, we examined the expression of inflammatory cytokines IL-8 and IL-33. The results showed that the expression of both IL-8 and IL-33 were significantly higher in psoriatic skin biopsy samples in comparison to the normal skin samples (Figure 4A-B).

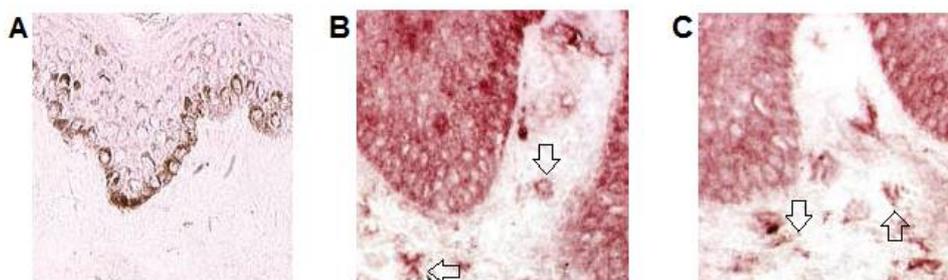
The upregulated expression of IL-8 and IL-33 was further confirmed by western blot in one psoriatic



**Figure 4:** Expression of (A) IL-8 and (B) IL-33 in normal and psoriasis skin biopsy samples determined by semi-quantitative RT-PCR analysis. Experiments were carried out in three biological replicates and presented as mean ± SD. The values were considered significant at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$



**Figure 5: (A)** Expression of IL-8 and IL-33 in normal and psoriasis skin biopsy samples determined by western blotting (B) quantification of IL-8 and IL-33 expression by densitometry. Experiments were carried out in three biological replicates and presented as mean ± SD. The values were considered significant at \* $p < 0.01$ , \*\* $p < 0.001$  and \*\*\* $p < 0.0001$



**Figure 6:** Immunohistochemistry showing distribution of IL-8 and IL-33 (A) normal skin biopsy sample showing no expression (B) psoriasis skin biopsy sample showing expression of IL-8 and (C) psoriasis skin biopsy sample showing expression of IL-33. Experiments were carried out in three biological replicates

and one normal skin samples and the protein expression of IL-8 and IL-33 correlated well with the results of RT-PCR data (Figure 5).

**Cell characteristics**

The results of immunohistochemistry revealed

the presence of positively stained cells in psoriatic skin tissue indicating the expression of IL-8 and IL-33. However, no background staining was observed in case of the normal skin tissues (Figure 6A-C).

## DISCUSSION

Psoriasis affects 3 % of the population around the world. In human, it is one of the prevalent T cell arbitrated inflammatory diseases. A highly elevated expression of cytokines has been reported in psoriasis which include but are not limited to IL-1, IL-6 and IL-12 [20]. CRH is an important stress induced hormone that has been reported to trigger immune response indirectly [21].

In the current investigation, we examined the expression of CRH and correlated its expression with CRHBP, IL-8 and the newly included cytokine IL-33. CRH plays an important role in the hypothalamic pituitary adrenal (HPA) axis response to stress. At the same time, it also acts indirectly in an anti-inflammatory response. In the current study, it was observed that expression of CRH was highly induced in psoriatic skin biopsy samples (Figure 1). The results are in agreement with the studies carried out previously wherein the expression of CRH was reported to be highly induced of psoriatic samples.

There is concrete evidence that the distribution and function of CRH is limited by the presence of CRHBP. Therefore, we evaluated the expression of CRHBP in both normal and psoriatic skin biopsy samples (Figure 2). It was observed that the mRNA expression of CRHBP was significantly low in case of psoriatic samples. These results suggested that the higher expression of CRH in psoriatic skin biopsy samples may be due to the unavailability of CRHBP. The expression of CRHBP was further confirmed by examining its expression at the protein level by western blotting. The results of western blots confirmed the mRNA expression determined by quantitative RT-PCR. Furthermore, it is believed that cytokines could be possible therapeutic targets for the treatment of psoriasis [22].

In the present study, we determined the expression of IL-8 and the new cytokine IL-33 in both the psoriatic and normal skin samples. The results suggested that mRNA expression of both the cytokines was highly elevated. This expression of IL-8 and IL-33 was further confirmed by western blotting. The results are in confirmation with previous studies wherein expression of several cytokines such as IL-1, IL-2, IL-6, IL-12 and IL-19 was found to be upregulated in psoriatic tissues [23]. While IL-8 was reported previously to be expressed in psoriasis [23], the present study represents one of the first studies that report the upregulation of IL-33 in psoriasis and IL-33 could therefore prove

to be an important therapeutic target. Based on the results of immunostaining, we found that mononuclear leucocytes are possible sources of IL-33 in psoriatic lesions. Taken together, the results suggest that IL-33 antagonists may prove to be important treatment options for the treatment of diseases like psoriasis.

## CONCLUSION

The findings of the present study reveal that the expression of CRH is up-regulated in psoriatic skin biopsy samples. This may be attributed to the down-regulation of CRHBP. Furthermore, the expression of CRH in psoriatic tissues is associated with concomitant accumulation of IL-8 and IL-33, indicating that CRH may trigger immune response in psoriasis by indirect stimulation of IL-8 and IL-33. Moreover, in view of the fact that IL-33 is highly expressed in psoriasis, IL-33 antagonists may prove an appropriate treatment option for psoriasis.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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