# Lysyl Oxidase Like -2 in the pathogenesis of psoriasis

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**Abstract: Objectives**: to investigate the role of Lysyl Oxidase Like -2 (*LOXL2*) in the pathogenesis of psoriasis. **Background: LOXL2** has been involved in gene transcription, cell motility/migration and adhesion, angiogenesis and differentiation, demonstrating their ability to affect both extra and intracellular cell functions.
**Patients and Methods:** twenty patients with psoriasis and ten healthy subjectsas a control group were included in this study. All patients were subjected to the following: History taking & clinical examination and dermatological examination and Punch biopsies were taken. each specimen was cut on routine slides for Hematoxylin and Eosin staining and immuno-staining with LOXL2. **Results:** Fifteen cases of psoriasis showed positive LOXL2 Immunoreactivity (75%).15 cases (100%) had cytoplasmic pattern. Comparing LOXL2 immunoreactivity in psoriasis versus normal epidermis showed downregulation of LOXL2 in psoriasis (75%) compared normal skin (100%). **Conclusion:** Downregulation of LOXL2 expression in psoriasis compared with normal skin. We identified novel *LOXL2* roles in tissue homeostasis and support it as a target for pathogenesis of psoriasis.
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## 1. Introduction

Psoriasis is a common, chronic relapsing/remitting immune mediated skin disease characterized by red, scaly patches, papules, and plaques, which often itchy **(1).** Psoriasis is associated with an overexpression of proinflammatory cytokines produced by Th1 cells and a relative underexpression of Th2 cytokines **(2).** The skin lesions found in psoriasis may differ in seriousness from minor limited patches to complete body coverage. The illness influences 2–4% of the general population **(3)**.

There are five primary types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic. Plaque psoriasis is the most widely recognized shape and ordinarily shows as red and white scaly patches on the top layer of the skin. Skin cells quickly collect at these plaque sites and create a silvery-white appearance (**3).**

The reasons for psoriasis are not completely understood. Psoriasis is not absolutely a skin lesion and can negatively affect numerous organ systems. Psoriasis has been related with an expanded danger of certain cancers, cardiovascular sickness and other immune-mediated disorders, for example, Crohn's illness and ulcerative colitis. It is generally considered a genetic disease thought to be activated or impacted by environmental factors **(1).** Psoriasis creates when the immune system mistakes a typical skin cell for apathogen, and conveys faulty signs that cause overproduction of new skin cells. It is not infectious. Oxidative anxiety, stress, and withdrawal of a systemic corticosteroid have each been proposed as a trigger for psoriasis **(4).** Damage to the skin can trigger local psoriatic skin changes known as the Koebner phenomenon **(5).**

No cure is accessible for psoriasis, however different medications can control the symptoms **(6,7)**.

Lysyl oxidase gene family includes five members acting as extracellular regulating enzymes: *LOX*, *LOXL*, *LOXL2*, *LOXL3*, and *LOXL4* **(8).** It is a copper-dependent amine oxidase that starts the covalent cross-linking of collagens and elastin in extracellular matrices (ECM) **(9).** *LOX* is discharged as a glycosylated proenzyme, handled by procollagen C-proteinase into a mature active form. *LOX* action regulation, due to either an increase or a decrease in its expression, induces various consequences for the structure and major characteristics of the ECM. *LOX* is essential in maintaining the characteristics of blood vessels and arteries, where its activity modulation is correlated to atherosclerosis, aneurism and human arterial dissection **(10).**

*LOX* down-regulation is related to numerous connective tissue issue seen in Ehler-Danlos disorder, cutis laxa, and Menke's disorder **( 11).** In tumors, *LOX* up-regulation is found in the stromal response saw around tumor foci in ductal breast carcinomas and in bronchopulmonary carcinomas **(12(.**

Lysyl oxidase like 2 (*LOXL2*), like its other LOX family members, has been accounted for to have amine oxidase action, however dissimilar to its family

members, its enzyme activity was not inhibited by beta-aminoproprionitrile (BAPN) **(13,14).**

Its overexpression in a number of cancers and its ability to promote epithelial to mesenchymal transition propose that *LOXL2* may play a role in tumor progression: expression is correlated with metastasis and diminshed survival in patients with aggressive breast cancer. Allosteric inhibition by AB0023 prevents development of the tumor microenvironment and diminishes metastatic tumor burden in xenograft models. However, inhibiting the enzyme activity of LOXL2 may not be adequate, since mutants that lack enzyme activity or inhibition of the activity by AB0023 antibody does not prevent inhibition of the differentiation of keratinocytes, promoting development of squamous cell carcinomas **(15,16).**

The aim of the present study is to investigate the role of Lysyl Oxidase Like -2 (*LOXL2*) in the pathogenesis of psoriasis.

## 2. Patients and Methods:

Twenty patients with psoriasis and ten healthy subjectsas a control group were included in this study. This study wasprospective and performed between Nov 2014 and Nov. 2016. The study was approved by Ethical Committee of Menoufia Faculty of Medicine; and informed consent was taken from each patient.

All patients were subjected to the following: History taking & clinical examination and dermatological examination.

Punch biopsies were taken under 2% lignocaine local anesthesia.

Specimens were fixed in 10% formalin solution, and then were sent to Pathology Department, Faculty of Medicine, Menoufia University, where they were submitted to routine tissue processing to be embedded in paraffin blocks. For each specimen, sections of 4μm thickness were cut on routine slides for Hematoxylin and Eosin staining to assess the pathological changes, while sections for immuno-staining with LOXL2 were cut on Poly L Lysine coated slides.

**Statistical analysis:**

Data were collected, tabulated and statistically analyzed using a personal computer with Statistical Package for Social Science (SPSS) version 15 program; Contingency tables were analyzed with the following tests:

**Descriptive statistics:**

**Qualitative data was expressed as:**

Number and percentage

**Quantitative data was expressed as:**

Arithmetic mean ($\bar{x}$), Standard deviation (SD), Percentage (%), Median, Range**.**

**Analytic statistics:**

**For comparing qualitative variables**

Chi- square test ($X^2$- test), Fisher's exacts test, Mann-Whitney U test (U test), Kruskal-Wallis test (K test), Pearson's correlation test (r- test).

## 3. Results

This retrospective case-control study was carried out on 30 subjects. These included 20 cases with psoriasis and10 age and gender matched control subjects (10 normal skin biopsies ).

**Immunohistochemial expression of LOXL2 in Normal skin**

All examined sections showed positive LOXL2 immunoreactivity. Expression percentage ranged from 10-50 with a mean±SD of 25.0±15.63. Intensity of expression varied from mild-moderate in 9 sections (90%), moderate-strong in 1 sections (10%). H score ranged between 10-110 with a mean±SD of 35.0±30.91. Regarding LOXL2 distribution, 8 sections (80%) had patchy distribution and 2 sections (20%) had diffuse distribution**.** Cytoplasmic localization occur in all sections**.** Positive stromal immunoreactivity was noted in all examined sections. **(Table 1 ).**

**Immunohistochemial expression of LOXL2 in psoriasis**

15 cases showed positive LOXL2 Immunore activity (75%).15 cases (100%) had cytoplasmic pattern. Expression percentage ranged from 2-50 with a mean±SD of 16.47±12.28. Intensity of expression varied from mild-moderate in 12 cases (80%) & moderate-strong in 3 cases (20%). H score ranged between 3-130 with a mean±SD of 31.53±33.80. Regarding LOXL2 distribution, 15 cases (100%) had patchy distribution. Positive stromal immunoreactivity was noted in 17 cases (85%) **(Table 2).**

**Comparison between marker expression in normal skin and psoriasis patients**

Comparing LOXL2 immunore activity in psoriasis versus normal epidermis showed down regulation of LOXL2 in psoriasis (75%) compared normal skin (100%). No significant differences regarding that LOXL2 expression (P=0.14), expression percentage (P= 0.21) and H score value (P=0.50) (P>0.05 for all). All slides show cytoplasmic localization (100%) in psoriasis and normal skin. **(Table3)**.

## 4. Discussion

The downregulation of *LOXL2* in psoriasis, demonstrated in the current study was not previously reported.

The positive immunoreactivity of *LOXL2* in psoriasis can be explained on basis of **Peinado et al. (17)** and **Moreno-Bueno et al. (18)** demonstrated that the implication of intracellular *LOXL2* in epithelial–mesenchymal transition (EMT) invasion and

metastasis through regulation of cell polarity and differentiation programs, dependent and independent of Snail1.

**Plate no. (1):** Sections examined from normal skin biopsy displays diffuse mild to moderate cytoplasmic LOXL2 expression ***(Immunoperoxidaseoriginal magnification 40XHPF).***

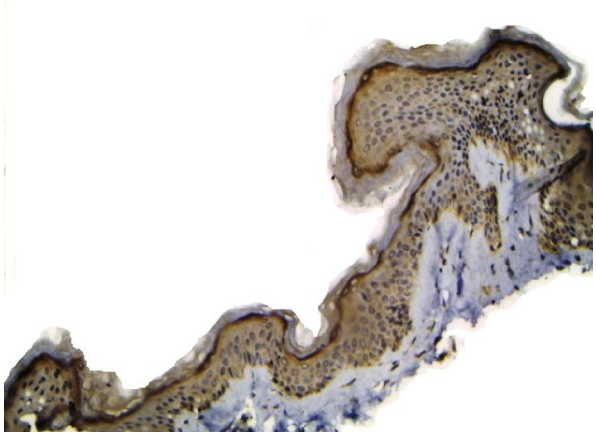

**Plate no. (2):** Mild to moderate cytoplasmic LOXL2 expression in the covering psoriatic epidermis ***(Immunoperoxidaseoriginal magnification 100 XHPF).***

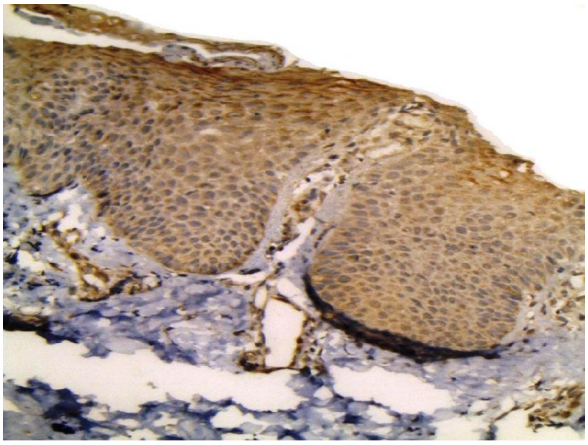

**Table (1)**: **Immunohistochemical expression of LOXL2 in normal skin.**

| **LOXL2 expression in normal skin** | **Normal skin N = 10** | |
|---|---|---|
| | **No** | **%** |
| **Expression** | | |
| Positive | 10 | 100 |
| Negative | 0 | 0 |
| **Intensity** | | |
| Mild – moderate | 9 | 90.0 |
| Mild strong | 0 | 0.0 |
| Moderate – strong | 1 | 10.0 |
| **Distribution** | | |
| Patchy | 8 | 80.0 |
| Diffuse | 2 | 20.0 |
| **Cellular localization** | | |
| Cytoplasmic | 10 | 100 |
| Other patterns | 0 | 0 |
| **Percent** | | |
| X ±SD | 25.0±15.63 | |
| Range | 10 – 50 | |
| **H score** | | |
| X ±SD | 35.0±30.91 | |
| Range | 10 – 110 | |
| **Stroma** | **No** | **%** |
| **Expression** | | |
| Positive | 10 | 100 |
| Negative | 0 | 0 |
| **Type** | | |
| Inflammatory | 8 | 80.0 |
| Adnexa | 0 | 0.0 |
| Adnexa & inflammatory | 2 | 20.0 |

**Table (2): Immunohistochemical expression of LOXL2 in psoriasis**

| | **Psoriasis N = 20** | |
|---|---|---|
| | **No** | **%** |
| **Expression** | | |
| Positive | 15 | 75.0 |
| Negative | 5 | 25.0 |
| **Intensity** | | |
| Mild – moderate | 12 | 80.0 |
| Mild strong | 0 | 0.0 |
| Moderate – strong | 3 | 20.0 |
| **Distribution** | | |
| Patchy | 15 | 100 |
| Diffuse | 0 | 0 |
| **Cellular localization** | | |
| Cytoplasmic | 15 | 100 |
| Other patterns | 0 | 0 |
| **Percent** | | |
| X ±SD | 16.47±12.28 | |
| Range | 2 – 50 | |
| **H score** | | |
| X ±SD | 31.53±33.80 | |
| Range | 3 – 130 | |
| **Stroma** | **No** | **%** |
| **Expression** | | |
| Positive | 17 | 85.0 |
| Negative | 3 | 15.0 |
| **Type** | | |
| Inflammatory | 15 | 88.2 |
| Adnexa | 0 | 0.0 |
| Adnexa & inflammatory | 2 | 11.8 |

**Table (3): Comparison between marker expression in normal skin and psoriasis patients**

| | **The studied groups** | | | | **Test of sig.** | P value |
|---|---|---|---|---|---|---|
| | **Normal skin N = 20** | | **Psoriasis N = 20** | | | |
| | **No** | **%** | **No** | **%** | | |
| **Expression** | | | | | | |
| Positive | 10 | 100 | 15 | 75.0 | FE | |
| Negative | 0 | 0 | 5 | 25.0 | 3.0 | 0.14 |
| **Intensity** | | | | | | |
| Mild – moderate | 9 | 90.0 | 12 | 80.0 | $X^2$ | |
| Mild strong | 0 | 0.0 | 0 | 00.0 | | |
| moderate strong | 1 | 10.0 | 3 | 20.0 | 6.49 | 0.17 |
| **Distribution** | | | | | | |
| Patchy | 8 | 80.0 | 15 | 100 | FE | |
| Diffuse | 2 | 20.0 | 0 | 0 | 3.26 | 0.15 |
| **Cellular localization** | | | | | | |
| Cytoplasmic | 10 | 100 | 15 | 100 | FE | |
| others | 0 | 0 | 0 | 0 | ----- | ------ |
| **Percent** | | | | | | |
| X ±SD | 25.0±15.63 | | 16.47±12.28 | | U | |
| Range | 10 – 50 | | 2 – 50 | | 1.25 | 0.21 |
| **H score** | | | | | | |
| X ±SD | 35.0±30.91 | | 31.53±33.80 | | U | |
| Range | 10 – 110 | | 3 – 130 | | 0.67 | 0.50 |
| **Dermal** | **No** | **%** | **No** | **%** | | |
| **Expression** | | | | | | |
| Positive | 10 | 100 | 17 | 85.0 | FE | |
| Negative | 0 | 0 | 3 | 15.0 | 1.67 | 0.53 |
| **Type** | | | | | | |
| Inflammatory | 8 | 80.0 | 15 | 88.2 | $X^2$ | |
| Adnexa | 0 | 0.0 | 0 | 0.0 | 0.34 | 0.61 |
| Adnexa & inflammatory | 2 | 20.0 | 2 | 11.8 | | |

Apart from the extracellular role of some *LOX* enzymes in the maturation of the ECM, previous studies have suggested novel functions in a wide spectrum of biological processes. specifically, *LOXL2* has been involved in gene transcription, cell motility/migration and adhesion, angiogenesis and differentiation, demonstrating their ability to affect both extra and intracellular cell functions **(19,20,21).**

In spite of the expanding proof supporting multiple *LOXL2* roles in tumorigenesis and metastasis, the mechanistic bases of these pathological functions and their relevance for future therapeutic interventions have not been completely settled. Also, in vivo information concerning *LOXL2* contribution in tissue homeostasis, the downstream effectors and its potential redundant action with other *LOX* members stay elusive**. ( 22).**

*LOXL2* was downregulated in head and neck squamous cell carcinoma (HNSCC) ( **23),** ovarian carcinoma **(24)** and lung adenocarcinoma **(25).**

This downregulation might be because of the localization of *LOXL2* in a chromosomal region that has been shown to be commonly deleted in a variety of human cancers. Studies on the function of *LOXL2* indicate that the gene is a candidate for tumor suppression **(25).**

Up-regulation of *LOXL2* mRNA and/or protein has been reported in breast, colon, esophageal, pancreatic carcinoma cell lines, and gastric cancer **(25).**

Additionally, the upregulation of the Notch1 signaling pathway recognized in papillomas got from mice, and the reverse scenario found in lesions, strongly supports a *LOXL2*- mediated action on this key-signaling pathway for epidermal differentiation during premalignant keratinocyte differentiation, hence impeding the maintenance of the differentiation status **(26).**

**Peinado et al**. **(17)** confirmed the ability of *Loxl2* to negatively modulate epidermal differentiation and the Notch1 signaling pathway in a more aggressive tumor context.

**Martin et al. (22)** showed the critical role of *LOXL2* in homeostasis of specific tissues and strengthen the potential value of *LOXL2* as a target for novel therapeutic interventions in squamous cell carcinoma. Germ-line deletion of *Loxl2* fatal in half of newborn mice mainly associated to congenital heart defects, while *Loxl2* overexpression evokes male sterility due to epididymal dysfunction caused by epithelial disorganization, fibrosis and acute inflammation. Remarkably, when challenged to chemical skin carcinogenesis, *Loxl2*-overexpressing mice increased tumor burden and malignant progression, while *Loxl2*-deficient mice exhibit the opposite phenotypes. *Loxl2* levels in premalignant tumors negatively correlate with expression of epidermal differentiation markers and components of the *Notch1* pathway. They showed that *LOXL2* is a direct repressor of *NOTCH1*. They identified for the first time novel *LOXL2* roles in tissue homeostasis.

**Conclusion:**

We showed that LOXL2 is doweregulated in psoriasis compared to normal skin.

We showed that *LOXL2* is a direct repressor of *NOTCH1*.

We identified novel *LOXL2* roles in tissue homeostasis and support it as a target for pathogenesis of psoriasis.

More studies are required to study role of *loxl2* in psoriasis.

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