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ORIGINAL ARTICLE

# Granulysin (GNLY) Gene Polymorphism and serum Interleukin 23 level in Psoriasis patients in Benha University Hospital

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## ABSTRACT

***Background:*** *Psoriasis is a chronic, non infectious, relapsing inflammatory skin disease caused by genetic, immunological and other factors in the environment, affecting 2–5% of the world's population . Granulysin is an anti-microbial peptide that contributes to local amplification of inflammation. The activation and maintenance of the T helper 17 pathway is the essential role of of IL23 in the pathogenesis of psoriasis.* ***Objectives:*** *Evaluation of the relation of granulysin (GNLY) gene single nucleotide polymorphism to psoriasis pathogenesis, measurement of interleukin23 (IL23) level in sera of cases of psoriasis and its relation to disease severity.* ***Methodology:*** *our study was done on 50 subjects divided into 2 groups:30 psoriasis patients, selected from Outpatients Clinics of Dermatology, Venerology and Andrology Department of Benha University Hospitals, and 20 apparent healthy control subjects of matched sex and age. Four ml venous blood samples were collected from psoriasis patients and control subjects to genotype GNLY rs7908 (C/G) and GNLY rs10180391(C/T) polymorphisms by Polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP)-based analysis and detection of interleukin (IL23) level using ELISA.* ***Results:*** *GNLY rs7908 CC genotype was significantly lower in psoriasis patients (6.7%) than in the control group (30%). The difference between patients and controls for GNLY rs10180391 allele frequencies was not statistically significant. IL23 level increased significantly with increased severity of psoriasis.* ***Conclusion:*** *GNLY rs 7908 gene polymorphism had a significant relation with severity of psoriasis. IL23 level was directly proportional to the severity of psoriasis.*

## INTRODUCTION

Psoriasis is a polygenic and multi factorial chronic inflammatory disorder mediated by different immune mechanisms and has a detrimental effect on psoriatic patients' life quality. The disease affects approximately 2-5% of the population[1]. Geographical areas with the highest prevalence were North America, Europe and Eastern Mediterranean, Asia, and Africa in descending order due to combination of genetic and environmental factors [2]. In Egypt, an estimated 500,000 persons have psoriasis disease. [3].

Granulysin is an anti-microbial peptide that contributes to local amplification of the inflammatory or immune response. It also acts as an alarming substance, with powerful chemo- attractant for T lymphocytes, monocytes, and NK cells, It can also attract and activate antigen presenting cells improving their ability to induce cytotoxic T lymphocytes (CTL) proliferation[4]. It has also been documented that granulysin is a ma jor Toll like receptor (TLR) ligand, a key factor in immune response and an essential modulator in pathogenesis of psoriasis.

In addition, granulysin was hypothesized to activate dendritic cells by releasing molecules associated with cell death, which include other alarmins, further further stimulating TLRs [5].These effectors activated by granulysin are known to be key players in the psoriasis immunopathogenesis [6].

The essential role of interleukin (IL)23 has been explained by other studies in psoriasis pathogenesis. It is a heterodimeric cytokine composed of two distinct subunits, p19 and p40. T-helper 17 pathway is activated and maintained by IL23 in the inflammatory process of psoriasis. Many studies showed that IL23 has a more significant role than other cytokines in the pathogenesis of psoriasis [7].

The present study aimed to to determine the relation of granulysin (GNLY) gene single nucleotide polymorphism with the pathogenesis of psoriasis and measure interleukin23 (IL23) level in sera of psoriasis patients in Benha University Hospital and its relation to disease severity.

## METHODOLOGY

The present study was performed in Medical Microbiology and Immunology Department and Dermatology, Venerology and Andrology Department Faculty of Medicine Benha University during the time (Janurary 2020 – April 2021).

The study was a case control study. It was done on 50 subjects. They were classified into two groups as follow:

- Cases including 30 psoriasis patients .They were selected from Outpatients Clinics of Dermatology, Venerology and Andrology Department of Benha University Hospitals. Patients were classified into groups according to the Psoriasis Area and Severity Index score (PASI). Accordingly, the patients were classified into mild (PASI<7), moderate (PASI 7–12) and severe psoriasis (PASI>12) [8] .
- The control included 20 apparent healthy individuals. They were almost matched for age and sex with the case group.

***Exclusion criteria:***

Patients with other skin diseases or with chronic diseases were excluded (other than hypertension and diabetes mellitus [DM]).

Control subjects who have a positive family history of psoriasis or other inflammatory and immune diseases were excluded.

The protocol of the study was explained to all subjects participated in our study and an informed consent was taken from all cases and control subjects. The ethical committee of the faculty of medicine Benha University gave its approval to the present study.

All patients were subjected to the following.

Full history taking, complete clinical examination, laboratory investigations:

**Sampling**:

Four ml venous blood samples were collected from psoriasis patients and control subjects; 2 ml in sterile EDTA containing tubes for DNA extraction, 2 ml in sterile plain tubes left for (10-20 minutes) at 37c˚, then centrifuged at 3000 rpm for 15 min to separate the serum which will be used for detection of interleukin (IL23) level [9].

**DNA extraction and genotype analysis**:

Genomic DNA was extracted from peripheral blood with the Kit of Biospin Whole Blood Genomic DNA Extraction (Bioflux), in accordance with the manufacturer's protocol.

- **Polymerase chain reaction restriction fragment length polymorphism (RFLP)-** based analysis was used to genotype GNLY rs7908 (C/G) and GNLY rs10180391(C/T) polymorphisms using PCR primers (Tables 1&2).
  PCR was performed in 0.2 ml PCR tubes containing 4µL genomic DNA, 2 µL primer (1 µL from each primer,10 µL master mix and 4 µL nuclease free water (total reaction volume 20 µL) .
- **Amplification conditions for the GNLY rs7908(C/G) polymorphism**: Initial denaturation at 95°C for 3 minutes, then 35 amplification rounds of (denaturation for 60s at 95°C, annealing for 90s at 56°C, and extension for 60s at 72°C), and finally 7-minute extension step at 72°C. The PCR products were checked on agarose gel, and then the products with 225 base pairs (bp)digested for one hour with restriction enzyme BbvCI (2 µL genomic DNA,1 µL restriction enzyme BbvCI, 5 µL NEbuffer and 22 µL nuclease free water).
- **Amplification conditions for the GNLY rs10180391 (C/T) polymorphism**: Initially denaturated at 95°C for 3 minutes, then 35 amplification rounds of (denaturation at 95°C for 60s, annealing at 55°C for 90s, extension for 60s at 72°C), and finally extension for 7-minute at 72°C. The products of PCR were checked on agarose gel for and then the products with 209 base pairs (bp) were digested for 15 minutes with restriction enzyme AseI (2 µL genomic DNA,1 µL restriction enzyme AseI, 5 µL NEbuffer and 22 µL nuclease free water).
- Gel electrophoresis was used to detect the products and viewed by staining with ethidium bromide and evaluated using a gel documentation system.

**Table 1: Sequence of primers and annealing temperature .**

| SNP ID | Primer sequencing | Annealing temp.$^{0C}$ |
|---|---|---|
| rs7908 | **F: 5´- TGT TCA GTA GGG TCA GGT GG-3´**<br>**R: 5´-GAT TCT GGA TCG AGG AAG CG-3´** | 56 $^{0C}$ |
| rs10180391 | **F: 5´- AAG CAA CAG AAG TCT CAG CC-3´**<br>**R: 5´-CTG CCA AGA GAG AAG CGA AC-3´** | 55 $^{0C}$ |

rs**:** reference SNP

**Table 2: SNP ID, restriction enzymes for detecting each single-nucleotide polymorphism (SNP),cutting sequencing, working temperature, incubation time and allele size(base pair)**

| SNP ID | Restriction enzymes | Cutting sequencing | Working temperature | Incubation time | Allele size (bp) |
|---|---|---|---|---|---|
| **rs7908** | BbvC1 | 5'-CC^TCAGC-3'<br>3'-GGAGT^CG-5' | 37°C | 60 minutes | GG: 225 bp<br>GC: 225, 183, 42 bp<br>CC: 183, 42 bp |
| **rs10180391** | Ase1 | 5'-AT^TAAT-3'<br>3'-TAAT^TA-5' | 37°C | 15 minutes | CC: 209 bp<br>CT: 209, 145, 64 bp<br>TT: 145,64 bp |

**Detection of IL23 level in serum samples using ELISA**:

IL23 level was measured in serum samples using Human IL-23 ELISA Kit (Sunredbio) in accordance with the manufacturer's protocol. 50µl per well of 96 pg/ml ,48 pg/ml ,24 pg/ml ,12pg/ml ,6 pg/ml standard solutions and 50µl streptavidin-HRP were added into the standard wells . Chromogen solution A and B, and stop solution were only added to blank well. 40µl serum sample, 10µl of IL-23antibody and 50µl streptavidin-HRP were then added to test wells. Then the sealing membrane was used, and gently shaked by hand, incubated 60 minutes at 37 ℃. The adhesive cover was removed carefully, and the liquid was drained and shaked away the remaining water. Then 50µl of chromogen solution A and 50µl of chromogen solution B were added to all wells. Gently mixed, incubated for 10 min at 37℃ away from light. 50µl of stop solution was then added into each well to stop the reaction . The optical density (OD) was measured under 450 nm wavelength which carried out within 15min after adding the stop solution using ELISA reader. A standard curve was drawn and IL 23 concentration in the samples was determined. The degree of color and the IL 23 level of samples were positively correlated.

## RESULTS

Among all studied psoriasis cases, 53.3% had mild, 33.3% had moderate and 13.3% had severe grades of psoriasis as shown in figure (1).

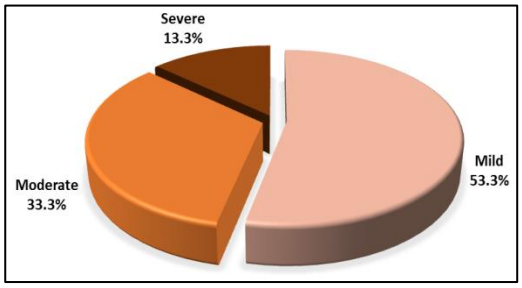


**Fig. 1**: Severity of studied psoriasis cases

GNLY rs7908; C allele had significantly lower proportions in cases when compared to control group ($p<0.05$ for each), with protective effect against development of psoriasis (OR<1 for each),as shown in table (3).

**Table 3. Distribution frequency of GNLY rs7908 genotypes and alleles within studied groups.**

| | | Control N=20 | | Cases **N=30** | | *p* | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | | **N** | **%** | **N** | **%** | | | |
| **rs7908** | ***GG*** | 3 | 15% | 15 | 50% | - | 1 | **Reference** |
| | ***GC*** | 11 | 55% | 13 | 43.3% | **0.047** | 0.422 | 0.180-0.990 |
| | ***CC*** | 6 | 30% | 2 | 6.7% | **0.006** | 0.194 | 0.060-0.623 |
| | ***GC+CC*** | 17 | 85% | 15 | 50% | **0.012** | 0.351 | 0.156-0.794 |
| | ***G*** | 17 | 42.5% | 43 | 71.7% | - | 1 | **Reference** |
| | ***C*** | 23 | 57.5% | 17 | 28.3% | **0.004** | 0.467 | 0.279-0.782 |

OR, odds ratio; CI, confidence interval.

No significant difference was found between patients and controls for GNLY rs10180391 allele frequencies, as shown in table (4).

**Table 4: Distribution frequency of GNLY rs10180391 genotypes and alleles within studied groups**

| | | Control N=20 | | Cases N=30 | | *p* | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | | | |
| **rs10180391** | ***CC*** | 9 | 45 | 12 | 40 | - | 1 | **Reference** |
| | ***CT*** | 8 | 40 | 13 | 43.3 | 0.753 | 1.131 | 0.525-2.434 |
| | ***TT*** | 3 | 15 | 5 | 16.7 | 0.793 | 1.149 | 0.408-3.237 |
| | ***CT+TT*** | 11 | 55 | 18 | 60 | 0.726 | 1.136 | 0.558-2.313 |
| | ***C*** | 26 | 65 | 37 | 61.7 | - | 1 | **Reference** |
| | ***T*** | 14 | 35 | 23 | 38.3 | 0.735 | 1.093 | 0.652-1.832 |

IL23 level is higher in cases (15.1-901.4pg/ml) than in control group (2.6-105.5pg/ml), as shown in figure(2).

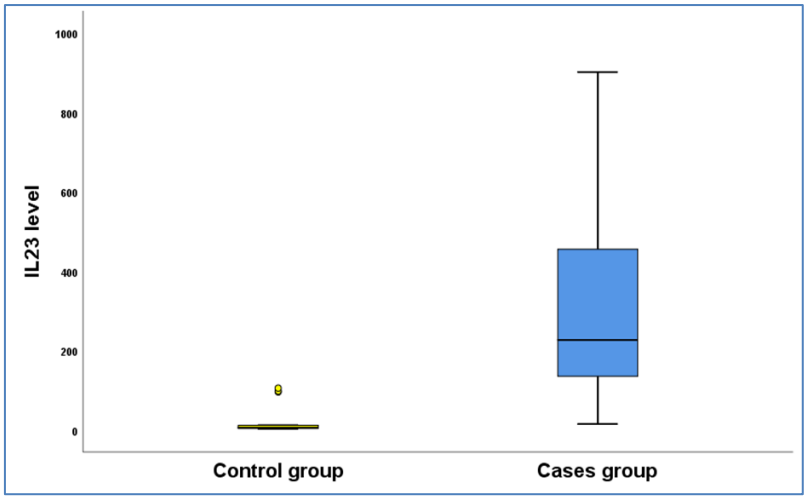


**Fig. 2: IL23 level among studied groups**

IL23 level increased significantly with increased severity (136.5, 429.6, 864.8 pg/ml in mild, moderate, severe respectively, p<0.001).as shown in table (5).

**Table 5. Correlation of IL23 level with severity.**

| | Mild | | Moderate | | Severe | | *p* |
|---|---|---|---|---|---|---|---|
| | Median | Range | Median | Range | Median | Range | |
| **IL23** | 136.5 | 15.1-318.9 | 429.6 | 233.5-510.6 | 864.8 | 755.2-901.4 | **<0.001** |

## DISCUSSION

Psoriasis is a chronic, multi system inflammatory disease, which primarily affects the skin, with a prevalence of around (2–5%)[1]. Its cause is a complex interaction between genetic, environmental, skin barrier injury, and immune disturbance[10]. Psoriasis may lead to multiple associated comorbidities [11].

The present study aimed to detect the relation of granulysin (GNLY) gene single nucleotide polymorphism to psoriasis pathogenesis and measure interleukin23 (IL23) level in sera of psoriasis cases in Benha University Hospital and its relation to disease severity.

The present study showed that among all studied cases, 53.3% had mild, 33.3% had moderate and 13.3% had severe grades of psoriasis.

With agreement to our study; Salgado-Boquete et al.[12] reported that among 254 patients, 45.7% have mild, 32.1% have moderate, and 20.2% have severe degree of psoriasis.

Our study is in contrast to Olejnik et al.[13] who found that among 127 patients; 96% were classified as mild, 12% as moderate, and 2% as severe.

The present study showed that; GNLY rs7908 CC genotype was significantly lower in the psoriasis patients than in control group. GC, CC genotypes and C allele had significantly higher proportions in the control group when compared to cases (P= 0.006 for CC genotype, P= 0.047 for GC genotype, P= 0.004 for C allele) with protective effect against development of psoriasis (OR=0.194 for CC genotype, OR=0.422 for GC genotype, OR=0.467 for C allele).

The present study is in agreement with Ermis et al.[14] who found a significant association between psoriasis and the GNLY rs7908 (C/G) polymorphism versus normal controls (OR=0.305; p=0.033 for CC genotype). OR=0.676; p=0.033 for GC genotype. OR=0.574; p=0.009 for C allele. CC genotype was significantly more frequent than GG genotype in the control group. This may be due to, in GNLY rs 7908 polymorphism, C/G nucleotide exchange occur in the protein coding region, leading to amino acid change from leucine to valine . This amino acid change caused by the rs7908 SNP can lead to decrease granulysin activity. Therefore, GNLY rs7908 C allele is thought to decrease the amount or activity of granulysin protein, leading to the complete elimination of trigger factors causing psoriasis[14].

Another study was made by Li et al.[15] detected the correlation between polymorphism in granulysin (GNLY rs11127) and effectiveness of treatment by pegylated interferon-alpha (PegIFNα) or nucleos(t)ide analogs (NUCs) in chronic hepatitis B patients . GNLY rs11127 was in significant association with combined response (CR) in patients who were treated with pegylated interferon-alpha (PegIFNα). The CR rate in patients with rs11127 CT or TT genotype was lower than that with CC genotype. This may be due to rs11127 SNP, located in the 4$^{th}$ exon of granulysin gene, changes the amino acid, which may influence the effectiveness of treatment by PegIFNα.

Our result was in disagreement with the results reported by Li et al.[15] who found that GNLY rs11127 had no significant association with combined response in nucleos(t)ide analogs -treated patients. This difference in the effectiveness of treatment by PegIFNα versus NUCs can be explained by various mechanisms of antiviral drugs.

The present study showed that distribution frequency of GNLY rs10180391 genotypes and alleles did not differ significantly within studied groups (p value >0.05).

This is in agreement with Ermis et al.[14] who found that distributions of genotypes of the GNLY rs10180391 were similar within studied group, and no relationship was detected between these groups (p > 0.05). GNLY rs10180391 allele frequencies were not statistically significant different within studied subjects (p = 0.604, OR = 1.114).

The present study is in disagreement with, Hou et al.[16] who found a statistically significant association between GNLY rs1866139 and GNLY rs11127 and hepatitis B virus infection. This can be explained by polymorphism in the GNLY rs1866139 and GNLY rs11127 are not effective in the activity or amount of protein [16].

Our study showed that IL23 level was significantly lower in the control group when compared to psoriasis patients (median=5.8 versus 226.9 pg/ml, p<0.001). IL23 level increased significantly with increased severity (p<0.001). IL23 level showed significant positive correlation with PASI score (rs=0.950, p<0.001).

This agrees with the studies performed by Kadhum et al. [8], Bilgiç et al. [17] and Fotiadou et al. [18] who reported a significant decrease in IL-23 level in the control group compared with the psoriasis patients. This may be due to the production of IL23 by keratinocytes and DCs which were activated by exposure to bacterial and fungal products that bind to TLRs [8].

On the other hand Filiz et al. [19] reported that IL-23 level was significantly higher in the control group compared to the patient group. This can be explained by that IL-23 was responsible for the initiation of lesions as a primary triggering mediator and that IL-23 was replaced by other cytokines later in the course of the disease.[20]

Our result is in disagreement with a study done by Bai et al. [21] and Ezhil [22] who found that IL-23 level was not significantly different between cases and control (P > 0.05). This may be due to the studied groups were of low socioeconomic level, and may have high levels of cytokines due to their lifestyle or the effect of exercise, type of food and body mass index that may affect the levels cytokines[22].

## CONCLUSION

GNLY rs 7908 gene polymorphism has a significant relation with the severity of psoriasis. Distribution frequency of GNLY rs10180391 genotypes and alleles did not differ significantly within studied groups. IL23 level was higher in psoriasis patients than in the control. IL23 level increased significantly with increased psoriasis severity.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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