

# The role of single nucleotide Interleukin-6 (IL-6) polymorphism gene in *Psoriasis vulgaris* patients at Haji Adam Malik Central Hospital, Medan-Indonesia



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## ABSTRACT

**Background:** Psoriasis is a chronic, recidive inflammatory skin disease. The main pathogenesis process is currently unknown. This disease is known as a complex disorder, influenced by both genetic susceptibility and environmental factors.

**Aim:** This study is aimed to analyze and determine the distribution of single nucleotide polymorphism (SNP) in IL-6 gene of psoriasis vulgaris patients and control patients.

**Method:** This study uses a case-control design to compare SNP in the IL-6 gene between psoriasis vulgaris group and control group.

**Results:** The most common genotype of the IL-6 gene Rs 1800795 in psoriasis vulgaris patients is GG (dominant homozygous) with a

total of 21 patients (46.7%). The most common genotype allele in the control group is Gc (heterozygous) with a total of 27 patients (60%). We observed a significant correlation between the groups ( $p = 0.044$ ).

**Conclusion:** Homozygote for IL-6 allele G (GG) is a prominent finding in the case group, while in the control group heterozygous allele (Gc) is a more common finding, thus suggesting that a patient with dominant homozygote alleles will have a more severe condition when compared to those who have the heterozygote alleles.

**Keywords:** single nucleotide polymorphism, psoriasis vulgaris, IL-6 gene

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## INTRODUCTION AND OBJECTIVES

Psoriasis is a recurrent chronic inflammatory skin disease. It can affect all age groups, and it is marked by red plaques covered by thick silvery-white scales with clear boundaries. Psoriasis lesions distributed symmetrically with predilection on the elbows and knees, scalp, lumbosacral region, buttocks and genitalia.<sup>1-3</sup>

The pathogenesis of psoriasis is a complex mechanism, and the main pathogenesis is still unknown. The excessive expression of skin inflammatory cytokines, especially type 1 cytokines such as interleukin (IL) -2, IL-6, IL-12, interferon (IFN) - $\gamma$  and tumor necrosis factor (TNF) - $\alpha$  in serum examination, is considered to be responsible for initiating and maintaining, also the recurrence of the skin lesions. On the opposite, decreased expression of anti-inflammatory cytokines such as IL-1, IL-4, and IL-10 could affect the regulation ability, and it is not in accordance with immune system in psoriasis.<sup>4-6</sup>

The certain etiology of psoriasis is unknown. However, there are many predisposing factors which play an important role as genetic predisposition and immunology disorders.<sup>7</sup>

Genetic factor has an important role in the etiology of psoriasis and studies have shown that many genes are involved in psoriasis. Currently, there are 12 vulnerable loci (positions of genes

on a chromosome) that have already identified (Psoriasis Susceptibility Locus (PSORS) 1 to 12). However, PSORS 1 is the only locus that has a high susceptibility rate.<sup>3</sup>

Abnormalities of a genetic factor in psoriasis may be associated with a single nucleotide polymorphism. It is a condition where there is a genetic variation, which manifests as a different single nucleotide base (Thymine (T) - Guanine (G) - Arginine (A) - Citocyn (C)) on certain position, in a sequence of DNA (deoxyribonucleic acid) which correlated with this disease.<sup>8</sup>

Patients with psoriasis express various pro-inflammatory cytokines such as interleukin, TNF, and IFN- $\gamma$ .<sup>13</sup> Interferon- $\gamma$  which produced by Th1 cells is important in the early phase of psoriasis. Interferon- $\gamma$  increases the migration of inflammatory cells and regulates a wide variety of other proinflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, IL-15, and TNF. These cytokines also inhibit apoptosis and increase proliferation of keratinocyte.<sup>14</sup> The expression of IL-6, which is detected by serum examination, is involved in the pathogenesis of various chronic inflammatory and autoimmune diseases, including psoriasis.<sup>9-11</sup>

The IL-6 gene Rs 1800795 in humans is located on the short arm of chromosome 7. In the promoter

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region (a DNA sequence) of the IL-6 gene, there are four polymorphisms; those are at position -597 (G/A), -572 (G/C), -373 (A/G) and -174 (G/C). The polymorphism -174 (G/C) affects the production of IL-6: G at position 174 (C to G transition at position -174) will increase production of IL-6, while the C at position 174 (G to C transition at position -174) will decrease the production of IL-6.<sup>12-14</sup>

The objective of this study is to analyze the role of SNP in IL-6 gene in the incidence of psoriasis vulgaris.

## PATIENT AND METHOD

### Subject

A total of 45 patients with psoriasis vulgaris and 45 controls were enrolled in this study. All subjects are between the age of 30-65 years. The examination was done in Immunodermatology Clinic, Dermatovenerology Department of H. Adam Malik Hospital. Demographic data of case and control group was adjusted. The inclusion criteria for subjects in the case group were patients with psoriasis vulgaris without a family history of psoriasis, patients with psoriasis vulgaris who do not suffer from fibrosis, chronic inflammation, malignancies and autoimmune diseases (systemic lupus erythematosus, Sjogren's syndrome, Churg-Strauss syndrome, idiopathic purpura thrombocytopenic and atopic dermatitis). For female patients, they should not be breastfeeding or pregnant. Subjects of the control group were healthy individuals with the same criteria to the case group.

### Tools and Materials

The tools used in this study were Invitrogen kit, Polymerase Chain Reaction (PCR) and Sequencer.

The materials used were 5 ml of venous blood, lysis buffer solution, buffer washers, buffer digestion, primer 50-GCC TCA ATGACG ACC TAA GC-30 and 50-TCA TGG GAA AAT CCC ACA TT-30, and NlaIII enzyme to cut DNA.

### Study Protocol

Five milliliters of blood were collected and then processed to isolate the DNA. The isolated DNA was then stored in Eppendorf tube at a temperature of -70°C. The next step was PCR by Restriction fragment length polymorphism (RFLP) technique. During PCR period, NlaIII enzyme was added aimed to cut the DNA. The final step was to read the results of PCR with agarose electrophoresis techniques.

### Trial Principle

RFLP technique is aimed at marking/separating a fragment of the genome containing important

genetic trait. RFLP analysis often used to detect genetic locations on chromosomes that encode an inherited disease or to detect the presence of gene diversity that associated with traits. This technique can detect mutations in noncoding regions of DNA and cause different cutting region by enzymes and can be separated by agarose electrophoresis gel. Different pieces or polymorphisms that generated will be passed down to next generations.<sup>15,16</sup>

Sequencing is the determination of base sequences in the DNA segments of relatively short DNA molecules. Nucleic acid sequencing is used to determine the genetic code of DNA molecule, or in another word, technique for determining the nucleotide sequences in DNA molecule, and known as DNA sequences.<sup>17</sup>

DNA sequencing uses PCR method as the foundation and base sequence (ACGT) of DNA that would be used as the template, then amplified by enzymes and similar materials to PCR. Interpretation by sequencer, electropherogram shaped like the up and down curve with different colors. Blue color indicates C base; red color indicates T base, black for G base, green for A base, and purple or light blue for N (error).<sup>18</sup>

### Data Processing and Analysis

Data were analyzed by using the computer system. Categorical data (nominal scale) was presented by displaying the frequency distribution and percentage.

Chi-Square test was used to test the hypothesis of the role of SNP IL-6 gene in psoriasis vulgaris, at the significance level of  $p < 0.05$ , by showing the Odds Ratio (OR), and a confidence interval of 95%.

If data is ineligible for Chi-Square test, an alternative test will be conducted using Fisher's Exact test.

### Ethical clearance

This study was conducted after obtaining ethical clearance from the ethics committee Faculty of Medicine, University of North Sumatera, No. 19/ KOMET/FK USU/2016.

## RESULT

The following demographic characteristics were observed from the study subjects: mostly women with 31 individuals (68.9%), with a mean age of 43.38 years old, and most study subjects are from Batak race, with 16 individual (35.6%).

Allele distribution of SNPs IL6 gene Rs1800795 between case and control group was shown. In the case group, there are 21 dominant homozygous individuals (46.7%). While in control group, there

**Table 1** Statistical analysis of individuals with genotype GG and Gc

Genotype	Case (n) (%)	Control (n) (%)	P	OR	95% CI
GG	21 (46,7)	11 (24,4)	<sup>a</sup> 0,044	2,577	1,016-6,528
Gc	20 (44,4)	27 (60%)			

<sup>a</sup>Chi-Square**Table 2** Statistical analysis of individuals with genotype Gc and cc

Genotype	Case (n) (%)	Control (n) (%)	P	OR	95% CI
Gc	20 (44,4)	27 (60)	<sup>b</sup> 1,000	1,296	0,333-5,039
Cc	4 (8,9)	7 (15,6)			

<sup>b</sup>Fisher exact**Table 3** Statistical analysis of individuals with genotype GG and cc

Genotype	Case (n) (%)	Control (n) (%)	P	OR	95% CI
GG	21(46,7)	11 (24,4)	<sup>b</sup> 0,156	3,341	0,801-13,943
Cc	4 (8,9)	7 (15,6)			

<sup>b</sup>Fisher exact

are only 11 individuals who are dominant homozygous (24.4%).

IL-6 gene Rs1800795 alleles with Gc genotype is classified as heterozygous. There are 20 heterozygous individuals (44.4%) in the case group and 27 individuals (60%) in control group.

The cc genotype is classified as homozygous recessive, and it can be found in four individuals (8.9%) in the case group and seven individuals (15.6%) in control group.

Analysis of the role of single genotype nucleotide polymorphism IL 6 gene Rs1800795 in psoriasis vulgaris is presented in the following tables:

## CONCLUSION

A dominant homozygous individual showed a single mutant allele on a chromosome. Generally, dominant homozygous individuals showed a more severe disease than individuals who are heterozygous.<sup>19</sup>

In genetic disorder, there is only a few of heterozygous individual that may present with a mild disease.<sup>20</sup>

The impact of a disease may result from genes interaction. Complex genetic abnormalities may result from a lot of polymorphisms which have two alleles or more. Polymorphism is often found in a complex disease which shares a common type; while some are specific for a certain disease. This is often found in inflammatory diseases mediated by immune system, which includes psoriasis vulgaris.<sup>20</sup>

A change of the sequence of nitrogenous bases in a DNA, which differs from normal variation is

called polymorphism mutation. Mutation can cause a disease or increase the risk of specific disease.<sup>16</sup> In an SNP, mutation that occurred is a point mutation as a result of transition process.<sup>21</sup>

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