Interleukin-23R (rs11209026) polymorphism confer the susceptibility in Psoriasis patients

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Abstract

Background: The recent advances in the understanding of psoriasis pathogenesis have clarified the pivotal role of interleukin (IL)-23. IL-23 is a heterodimeric cytokine, consisting of two subunits, the unique p19 and p40, which are shared with IL-12. The basic role of IL-23 in psoriasis is the activation and maintenance of the T-helper17 pathway. IL23R is a key player in the proliferation and survival of Th17 cells. The IL23 axis is the protagonist of the immunopathogenesis of psoriasis. Therefore, present study suggests to evaluated and investigated the single nucleotide polymorphisms of IL-23R (rs11209026) gene in seventy subjects. This study was find out the significant importance of IL-23R rs11209026 in disease susceptibility.

Material and Methods: A blood sample was collected from two groups. The first group was patients with Psoriasis; the second group was healthy volunteers. Blood of this sample was collected in ethylinditetracitic acid (EDTA) tube and stored at 4°C for DNA extraction, polymerase-chain reaction Restriction Fragment Polymorphism in length (PCR-RFLP) method which used to study SNP of IL-23R (rs11209026) gene.

Results: The current study showed the range age of patients was (14–60), the results also revealed that 45.7% of the patients were males, and 54.3% of the patients were females. Regarding the IL-23R gene rs11209026 single nucleotide polymorphism in Iraqi psoriasis patients frequency distribution of genotype GG was significantly more frequent in patients group than in control, Patients were distributed as 7 (10.0 %) with allele A and 63 (90.0 %) with allele G; whereas, control group included 21 (30.0 %) individuals with allele A and 49 (70.0 %) subjects with allele G.

Keywords: Psoriasis, IL-23R (rs11209026), Allele, Genotype, RFLP-PCR

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Introduction

Psoriasis is a chronic inflammatory, immune-mediated skin disorder that is characterized by a complex pathophysiology. It synergistic influence by genetic and environmental factors along with the interplay of innate and adaptive immunity eventually leads to the abnormal keratinocyte proliferation and formation of the psoriatic lesions (1). The worldwide prevalence is about 2%, but varies according to regions (2). It shows a lower prevalence in Asian and some African populations, and up to 11% in Caucasian and Scandinavian populations (3) (4). Psoriasis severity is often categorized as mild, moderate, and severe, which is guided by measurements such as body surface area (BSA), Physician’s Global Assessment (PGA), and the Psoriasis Area and Severity Index (PASI) (5). Many different guidelines and consensus definitions of psoriasis severity exist and include a combination of assessor- and patient-reported measures for disease classification. The exact etiology is unknown, but it is considered to be an autoimmune disease mediated by T lymphocytes. There is an association of HLA antigens seen in many psoriatic patients particularly in various racial and ethnic groups. Familial occurrence suggests its genetic predisposition.
Environmental factors appear to induce inflammatory diseases in individuals with latent genetic susceptibility. Many alterations could be triggered by environmental factors such as diet, microbial infections (from bacteria, fungus and virus), chemical irritants or UV radiation exposure (6) and bad habits (such as smoking and drinking) (7) (8). The immunopathogenesis of psoriasis involves complex interactions between the innate and the adaptive immune system. In the early development of lesions, it appears to be essential the activation of the plasmacytoid dendritic cells (pDC), producing INF-a (9)(10). INF-a and other proinflammatory cytokines promote an excessive activation of the myeloid dermal dendritic cells (mDC), whose number is much increased in psoriatic plaques (11)(12). The mDC, in turn, are sources of various cytokines (IL-23, TNF-α, IL-12, IL-6, IL-1 and IL-20) and end up capturing a protein antigen (Ag) in the dermis not yet defined (11)(12). IL-23 is produced by antigen-presenting cells, such as dendritic cells and monocytes/macrophages upon activation and initiates signaling by binding to IL-23R, which in return increases the expression of RORt and IL-17 via STAT3 (13). The basic role of IL-23 in the pathogenesis of psoriasis has been clarified, and it is associated with the biology of the Th17 lineage. The initial differentiation of naïve T lymphocytes to Th17 requires the presence of TGF-β, IL-6, and IL-1β, while IL-23 is necessary for the activation and maintenance of Th17 in order to secrete the pro-inflammatory cytokines IL-17, IL-22, IL-21, and tumor necrosis factor alpha, which eventually contribute to the formation of the psoriasis (14) (15). The IL-23 receptor (IL-23R) gene is located on chromosome 1 (1p31). The effects of IL-23 on T-cells are mediated via a receptor complex consisting of an IL-12Rβ1 and a specific IL-23R chain. IL23R is a key player in the proliferation and survival of Th17 cells. IL-23R transcripts are, however, found in bone marrow and in various T-cell subsets (16). Factors increasing IL23R mRNA expression include IL-23 itself, IL-6, IL-21, transforming growth factor (TGF)-β and TH17 cell activation. Among the determinants that decrease transcriptional activity of IL23R is the TH1-type cytokine IFNγ that simultaneously increases IL12Rβ2 mRNA expression (17).

Materials and Methods
A case control study was conducted in AL-Diwaniyah province. Based on 35 patient with psoriasis which include (16 male and 19 female), who attended the consultant clinic for dermatology in AL-Diwaniya teaching hospital in the period from 10 September 2019 to 20 February 2020 under the supervision of dermatology physician, were included in this study. Patients were diagnosed with psoriasis according to history, clinical examination in addition to that the information about each case was collected from patients in the hospital according to the form mentioned in the appendix. In addition to that, about 35 (16 male and 19 female) healthy volunteers were included as a control group. Blood samples were collected by venipuncture from 35 patients and their healthy controls, 2.5 ml of venous blood were drawn by disposable syringe under aseptic technique. A sample of blood was collected in ethylditetraacetic acid (EDTA) tube and stored at 4°C for DNA extraction for detection of IL-23R gene polymorphism. The genotypes of the IL-23R (rs11209026) gene were determined by Restriction Fragment Length Polymorphism (RFLP)-PCR, Table (1). Genomic DNA from blood samples were extracted by using G-spin DNA kit extraction kit (Frozen Blood) INtRON, Korea, and done according to company. The PCR products were amplified using a Maxima PCR PreMix (iNtRON), and then the PCR products were visualized in an ethidium bromide-stained 2% agarose gel using a UV Transilluminator.

Statistical analysis
Chi-square test assumption was assessed for both the patient and control groups by comparing the observed numbers of each genotype. Data were presented, summarized and analyzed using two software programs. These were the Statistical Package for Social Science (SPSS) version 23 and Microsoft Office Excel 2016. Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the association.
between the genotypes, alleles or haplotypes and the risk of ALL. The results are presented as the mean values ± 1 standard deviation (SD), and a P value of ≤0.05 was considered to indicate statistical significance.

**Results**

Distribution of IL-23R (rs11209026) polymorphism was detected by RFLP-PCR technique, at this locus there are two genotype GG, and GA figure (1).

![Figure (1): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of IL-23R gene polymorphisms (rs11209026) using Hpy188I restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygote wild types alleles, the products were digested by restriction enzyme into 250bp, 65bp, and 35bp bands and lane (G/A) heterozygote, the products were digested by restriction enzyme into 285bp, 250bp, 65bp, and 35bp bands](image)

The mean age of patients was 38.60 ±12.67 years and that of control subjects was 38.91 ±12.82 years; the difference in mean age between both groups was not significant \((P = 0.918)\), which ensures age matching that is mandatory for such a study. The frequency distribution of patients and control subjects according to age is also shown in table (2).

**Table (1):** primers sequence with orientation and the PCR product size

<table>
<thead>
<tr>
<th>IL-23R gene (rs11209026)</th>
<th>Primer</th>
<th>Sequence</th>
<th>PCR product size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>AGTCACTCTGTGGCCTAAAGTAAAG</td>
<td>350bp</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AGATTTTTCTAGTAAACACTGAAATGA</td>
<td></td>
</tr>
</tbody>
</table>

**Table (2):** The Patient-Control Difference in mean age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control group (n = 35)</th>
<th>Study group (n = 35)</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>38.60 ±12.67</td>
<td>38.91 ±12.82</td>
<td>0.918 NS</td>
</tr>
<tr>
<td>Range</td>
<td>14 -60</td>
<td>14 -60</td>
<td></td>
</tr>
<tr>
<td>&lt; 20, (n)</td>
<td>4 (11.4 %)</td>
<td>4 (11.4 %)</td>
<td></td>
</tr>
<tr>
<td>20-40, (n)</td>
<td>17 (48.6 %)</td>
<td>16 (45.7 %)</td>
<td></td>
</tr>
<tr>
<td>&gt; 40, (n)</td>
<td>14 (40.0 %)</td>
<td>15 (42.9 %)</td>
<td></td>
</tr>
</tbody>
</table>

\(n\): number of cases; \(SD\): standard deviation; I: Independent samples t-test; C: Chi-square test; NS: not significant at \(P > 0.05\)

Regarding to gender distribution about 16 patients (45.7 %) were male, and 19 patients (54.3 %) were females, while control subjects were selected to match patients group; and there was no significant difference in frequency distribution according to gender \((P = 1.000)\), which ensures gender match that is mandatory for such a study. The frequency distribution of patients and control subjects according to gender is shown in table (3).
The current study revealed that frequency distribution of patients with psoriasis and control subjects according to IL-23R genotypes is shown in table (4). Patients were distributed as 7 (20.0 %) with heterozygous GA genotype and 28 (80.0 %) with homozygous GG; whereas, control group included 21 (60.0 %) individuals with heterozygous GA genotype and 14 (40.0 %) subjects with homozygous GG. There was highly significant difference in the Frequency distribution of patients with psoriasis and control subjects according to IL-23R genotypes ($P = 0.001$), GG is a risk factor with an OR of 6 (2.06-17.48, 95% confidence interval). Frequency distribution of patients with psoriasis and control subjects according to IL-23R alleles is shown in table 5. Patients were distributed as 7 (10.0 %) with allele A and 63 (90.0 %) with allele G; whereas, control group included 21 (30.0 %) individuals with allele A and 49 (70.0 %) subjects with allele G. There was highly significant difference in the Frequency distribution of patients with psoriasis and control subjects according to IL-23R alleles ($P = 0.003$). The A allele is protective while G allele is a risk factor with 3.86 OR (1.52-9.81, 95% Confidence interval) and etiologic fraction 0.42.

Table (4): Frequency distribution of patients with psoriasis and control subjects according to IL-23R genotypes

<table>
<thead>
<tr>
<th>IL-23R Genotype</th>
<th>Patients n = 35</th>
<th>Control n = 35</th>
<th>$P$</th>
<th>OR</th>
<th>95% CI</th>
<th>EF</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>7 (20.0 %)</td>
<td>21 (60.0 %)</td>
<td>0.001 C</td>
<td>0.17</td>
<td>0.06-0.49</td>
<td>---</td>
<td>0.56</td>
</tr>
<tr>
<td>GG</td>
<td>28 (80.0 %)</td>
<td>14 (40.0 %)</td>
<td>HS</td>
<td>6.00</td>
<td>2.06-17.48</td>
<td>0.56</td>
<td>---</td>
</tr>
</tbody>
</table>

Table (5): Frequency distribution of patients with psoriasis and control subjects according to IL-23 alleles

<table>
<thead>
<tr>
<th>IL-23 allele</th>
<th>Patients n = 70</th>
<th>Control n = 70</th>
<th>$P$</th>
<th>OR</th>
<th>95% CI</th>
<th>EF</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 (10.0 %)</td>
<td>21 (30.0 %)</td>
<td>0.003 C</td>
<td>0.26</td>
<td>0.10-0.66</td>
<td>---</td>
<td>0.42</td>
</tr>
<tr>
<td>G</td>
<td>63 (90.0 %)</td>
<td>49 (70.0 %)</td>
<td>HS</td>
<td>3.86</td>
<td>1.52-9.81</td>
<td>0.42</td>
<td>---</td>
</tr>
</tbody>
</table>

Dissection

In this study, we selected the IL-23 receptor (IL-23R) as a candidate gene. The IL23R gene is located on chromosome1p31 and the encoded protein forms a receptor for IL-23 together with the b1 subunit of IL-12 (IL12Rb1) (18). IL-23R expression on memory T cells, natural killer T cells, monocytes, and dendritic cells correlates with cellular responsiveness to IL-23 (18). Polymorphisms in IL23R have also been found to associate with the risk of psoriasis in many studies (19-20). Recently, a genome-wide association study (GWAS) yielded single-nucleotide polymorphisms (SNPs) within IL23R that conferred a risk of psoriasis, but the results are inconclusive (19-21). Current results confirm an association between genes in the IL-23R pathway with psoriasis. The GG genotype at the rs11209026 locus was reported to increase the risk of psoriasis (22). The IL-23R gene rs11209026 G minor allele frequency was reported to be significantly higher in psoriatic patients (23), rs11209026 polymorphism was reported to be associated with psoriasis in Europeans, although this polymorphism was not associated with psoriasis in the Asians (24). The rs11209026 G allele was reported to be a risk factor for psoriasis in some populations (23-24). The present results agree with results of previous studies such as a study done by Cargill et al, 2007 they identified a common risk haplotype was for each gene (25). Another study mentioned that there is significant association between polymorphism of IL-23Rrs11209026 and susceptibility to psoriasis in population. 

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Health Medicine & Public Annals of Tropical (26), this emphasizes the concept that in complex genetic disorders, the emergence of functional genetic variants can decrease, as well as increase, disease susceptibility. However, according to study done by Cabaleiro et al., 2013 in Spanish populations, they did not find any association between psoriasis polymorphism of IL-23Rrs11209026 (27). Another study done by FILIZ et al., 2019 the distribution of genotypes and alleles at the rs11209026 locus (GG, GA) was also not significant, and the AA genotype was not found in the patient or the controls group, since the rs11209026 AA polymorphism has not been studied in our country, we were unable to compare our results with published data from the same population (28). Polymorphisms may vary depending on diseases and races. Thus, while the association of polymorphisms with a disease is not significant in one race, it may be a risk factor for the same disease in a different race. Like other candidate gene studies, there are several limitations in current study. As the power to detect disease susceptibility genes is influenced by the number of the patient's sample, in current study seemed to be relatively small. Moreover, interactions of many different genes, polymorphisms, and environmental factors contribute to the development of disease process. Finally, current results suggest that IL-23 receptor GG genotype variant of rs11209026 would be contribute to psoriasis susceptibility. Future studies will systematically explore genetic commonalities between inflammatory barrier diseases in general (29). Fine mapping of the IL23R-linked region shows variants segregating at IL23R coding and flanking regions significantly associated with psoriasis. In particular, there are extended haplotypes in this region that protect against psoriasis susceptibility. Importantly, it also appears that at least two IL23R polymorphisms, P310L and R381Q (30), independently contribute to linkage disequilibrium with the psoriasis phenotype. In addition, both sliding window haplotype analyses and longer range haplotype work pinpointed the association signal to the IL23R coding region. This is particularly important as the interleukin 12 receptor subunit-encoding genes, IL12RB2, is located 47 kb from the 30 end of IL23R and some SNP pairs exhibit substantial and even perfect linkage disequilibrium between sites located in the coding regions of the two genes. Recent animal studies show that the IL12RB2 knockout mouse develops an autoimmune/lymphoproliferative disorder with aberrant IL-12 signaling (31). The HapMap project has general population genotype data on both missense SNPs, rs7530511 (P310L) and rs11209026 (R381Q) (32) At rs11209026, the A allele (minor allele) was found on CEU and YRI chromosomes (8 out of 120 CEU chromosomes and 2 out of 120 YRI chromosomes), but not on chromosomes from the two East Asian samples (CHB and JPT). Hence, it is possible that rs11209026 may predispose some African and/or African-derived populations to autoimmunity and autoinflammatory traits, particularly if the effect size is larger in those subpopulations than European-derived samples.

Conclusion
There are significant association between IL-23R gene rs11202690 polymorphism and susceptibility to psoriasis. Moreover, the G allele is risk factor, while A allele is protective.

Conflict of interest: There is no conflict of interest

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References


