Genetic polymorphisms of \(NF\kappa B1\) -94 del/ins ATTG, \(NF\kappa B1A\) 2758 A>G and \(SUMO\) rs237025 G>A in psoriasis


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Abstract

Background: Nuclear factor-\(\kappa B\) (\(NF\kappa B\)) and small ubiquitin-like modifier (\(SUMO\)) are key transcription factors involved in the regulation of immune responses and apoptosis. The aim of this study is to test for the association of \(NF\kappa B\) and \(SUMO\) gene polymorphisms with the susceptibility and severity of psoriasis among Saudi cases.

Subjects and Methods: This is a case controlled study including 85 Saudi psoriasis patients in addition to 92 matched healthy unrelated controls from the same locality. For all participants, DNA was analyzed by PCR for characterization of \(NF\kappa B1\) ins/del, \(NF\kappa B1A\) A>G and \(SUMO\) rs237025 G>A gene polymorphisms.

Results: Compared to controls, psoriasis patients showed a non-significant difference for all frequencies of genotypes and alleles of \(NF\kappa B1\) ins/del, \(NF\kappa B1A\) A>G and \(SUMO\) G>A polymorphisms (\(p>0.05\)). However, cases with the plaque type had significantly higher frequency of the \(SUMO\) A allele carriage (GA+AA genotypes) than the guttate type (78.6% vs. 21.4%, \(p=0.02\)). The PASI score was also significantly higher among cases with the \(NF\kappa B1A\) AA genotype than other cases (\(p=0.00\)).

Conclusion: Genetic polymorphisms of \(NF\kappa B1\) -94 ins/del ATTG, \(NF\kappa B1A\) 2758 A>G and \(SUMO\) rs237025 G>A were not associated with the susceptibility to psoriasis vulgaris in Saudi patients. However, it might be associated with the expressivity of the disease in terms of its clinical type and severity.

Keywords: Psoriasis; Gene polymorphism; Psoriasis; \(NF\kappa B\); \(SUMO\).

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Introduction

Psoriasis vulgaris is a common chronic inflammatory skin disease characterized by red scaly plaques with various degrees of severity.\(^{[1]}\) The pathogenesis of psoriasis has been speculated to be due to factors originating in the skin, immune system or in the human genome.\(^{[2-4]}\) The immune basis of psoriasis was manifested by the presence of the activated type 1 T cells (Th1) and their cytokines in psoriatic lesions.\(^{[5-8]}\) As an important transcription factor, NFκB mediates the survival response by inhibiting p53-dependent apoptosis and up-regulating anti-apoptotic members of the Bcl-2 family and caspase inhibitors.\(^{[9,10]}\) Thus, NFκB activation might induce resistance to apoptosis of peripheral blood mononuclear cells in patients with autoimmune diseases.\(^{[11]}\) NFκB was found to augment the transcription of crucial genes in the activated Th1 cells which were involved in the pathogenesis of psoriasis such as TNF-α, IL-8, IL-12 and cyclin D.\(^{[5-8,12-14]}\) NFκB designates a group of critical transcription factors, the major form of which is a heterodimer of the p50 and p65/Rel A subunits, encoded by the genes NFκB1 and NFκB2, respectively.\(^{[15]}\) NFκB 1 maps to chromosome 4q23–q24 and consists of 24 exons,\(^{[16,17]}\) and its inhibitory gene NFκBIA (encoding for IκB) is located on chromosome 14q13 and is including six exons.\(^{[18,19]}\)

Genetic studies have identified single nucleotide polymorphisms (SNPs) in NFκB1 and NFκBIA.\(^{[20,21]}\) Recently, a common insertion/deletion (-94 insertion/deletion ATTG rs28362491) polymorphism in the NFκB1 promoter region and a 39 untranslated region (39UTR) polymorphism 2758 A>G (rs696) in NFκBIA were observed to be significantly correlated with inflammatory bowel disease\(^{[22,23]}\) and cancers.\(^{[24,25]}\) On the other hand, SUMO family (SUMO1, 2, 3 and 4) was found to act as a negative regulator of NFκB through the inhibition of IκBa degradation.\(^{[26,27]}\) The SNP rs237025 A>G in SUMO4 gene (methionine/valine, M55V) was shown to enhance NFκB transcriptional activity and some its dependent genes.\(^{[28,29]}\)

Previous studies have verified that this polymorphism was strongly associated with type1 diabetes,\(^{[30]}\) rheumatoid arthritis (RA),\(^{[31,32]}\) systemic lupus erythematosus (SLE),\(^{[33]}\) Behcet’s disease\(^{[34,35]}\) and Vogt–Koyanagi–Harada syndrome (VKH).\(^{[36]}\) Therefore, it was also speculated to be associated with psoriasis.\(^{[37-42]}\)

This study is aiming to test for the association between polymorphisms in NFκB1, NFκB IA and the SUMO4 with psoriasis vulgaris susceptibility and clinical pattern among Saudi patients.

Subjects and methods

Eighty five Saudi psoriasis patients were enrolled into the study. They included 45 males and 40 females with a mean (± SD) age of onset of the disease of 23.6 (±11.4) years. Controls were in the form of 47 males and 45 females of a mean (± SD) age of 26 (±11.3) years. Cases were recruited from the Dermatology Clinic affiliated to Qassim University, Saudi Arabia. All the patients were diagnosed as typical cases of psoriasis by a consultant dermatologist. For all patients, data related to their age, sex, age of onset, family history of psoriasis, consanguinity pattern, clinical presentation in terms of the area involved and severity index (PASI) score were recorded. The controls were selected from healthy blood donors with no past or family history of immune or dermatologic disorders. The work started after obtaining an authorized approval from the local health authorities as well as an informed consent from all participants.

For all patients and controls, DNA was extracted from peripheral blood samples and purified using the MagNa Pure purification system (Roche, Berlin, Germany). For determination of the NFκB 1 promoter (rs28362491) polymorphism, the SNP-containing fragment was amplified using the following primers: 5'-TGGGCACAAGTCTGTATGA-3' (forward) and 59-CTGGAGCCGTAGGGAAG-39 (reverse).

PCR was run at 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s with a final extension at 72°C for 10 min. The PCR products (281/285 bp in size) were digested with PfilMI (Fermentas, Vilnius, Lithuania) at 37°C overnight followed by 2% agarose gel electrophoresis.\(^{[43]}\) For determination of the NFκB IA G/A substitution in 3' untranslated region (rs696) polymorphism, the SNP containing fragment was amplified using the following primers: 5'-
Statistical analysis

All data were analyzed using SPSS version 12.0.1 software. To test for the association of the studied genetic variants and susceptibility to psoriasis, the chi-square, Fisher exact and odds ratio tests were used to compare genotype and allele frequencies of psoriasis patients and controls. Association of genetic variants to the clinical pattern and severity of psoriasis was carried out by comparing the frequency of genotypes of case-subgroups regarding their age of onset, gender, family history, PASI score and consanguinity. Hardy Weinberg equilibrium was tested separately for patient and control groups comparing the observed vs. expected frequencies of genotypes. All statistical tests were two-sided, and statistical significance was considered positive at a p value <0.05.

Results

Comparing cases to controls regarding the frequencies of their NF-κ B1-94 ins/del ATTG, NF-κB1A A>G and SUMO4 G>A polymorphic variants (Table 1) showed no statistical significance (p>0.05) both in the recessive and dominant models. Although statistically non-significant (p>0.05), cases had higher frequencies of certain genotypes compared to controls as SUMO4 GG genotype (25% vs. 19.6%), NF-κ B1 ins/del (48.8% vs. 41.3%) and NF-κB1A GG genotype (29.8% vs. 23.8%). Both cases and controls were conforming to the Hardy-Weinberg equilibrium manifested by of a non-significant difference (p>0.05) between the observed and expected genotype frequencies of all three studied polymorphisms (Table 1). Comparison of the genotype frequencies among cases-subgroups related to their age of onset, gender, consanguinity, family history, PASI score and type of lesion is shown in Table 2. All subgroups showed a non-significant difference (p>0.05) in their genotypic frequencies except for cases with the plaque type that showed significantly increased frequency of SUMO4 A allele carriage (GA+AA genotypes) than cases with the guttate type that showed a higher frequency of the GG genotype (p=0.02). The PASI score was also found significantly higher among cases with NF-κB1A AA genotype (p=0.00).

Table 1. Polymorphism of NF-κ B1-94 ins/del ATTG, NF-κB1A A>G and SUMO4 rs 237025 G>A among cases of psoriasis compared to controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>p</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NF-κ B1-94 ins/del</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ins/ins</td>
<td>30 (35.7)</td>
<td>33 (41.2)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>ins/del</td>
<td>41 (48.8)</td>
<td>33 (41.3)</td>
<td>0.46</td>
<td>1.4(0.7-2.7)</td>
</tr>
<tr>
<td>del/del</td>
<td>13 (15.5)</td>
<td>14 (17.5)</td>
<td>0.85</td>
<td>1.0(0.4-2.5)</td>
</tr>
<tr>
<td>ins/del+ del/del</td>
<td>54 (64.3)</td>
<td>47 (58.8)</td>
<td>0.57</td>
<td>1.3 (0.7-2.4)</td>
</tr>
<tr>
<td>ins allele</td>
<td>101(60.1)</td>
<td>99 (61.9)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>del allele</td>
<td>67(39.9)</td>
<td>61(38.1)</td>
<td>0.83</td>
<td>1.1(0.7-1.7)</td>
</tr>
<tr>
<td>HWE</td>
<td></td>
<td></td>
<td>χ²=0.03, p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>NF-κB1A A&gt;G</strong></td>
<td></td>
<td></td>
<td>χ²=1.3, p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>7 (8.3)</td>
<td>12 (15.0)</td>
<td>Ref</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

This study is an attempt to shed a light on the genetic background of psoriasis among Saudi subjects which is obviously supported by the finding of a positive consanguinity and family history of the disease among about 1/5 of patients. To the best of our knowledge, this is the first study testing for the association between polymorphisms in NF-κB1, NF-κB1A and the SUMO4 genes with psoriasis vulgaris susceptibility and clinical pattern among Saudi patients. Although statistically non-significant, cases had higher frequencies of certain genotypes compared to controls as NF-κB1 ins/del, NF-κB1A GG and SUMO4 GG genotypes. This in fact might attract our attention to the potential effect of the relatively small sample size of the study on the power and significance of results. In this respect we would recommend undertaking another large scale study testing for these genetic polymorphisms along with their expression pattern in the psoriatic skin cells. Regarding the clinical pattern and severity of psoriasis, we have observed that NF-κB1A GG was associated with higher PASI score while SUMO4 AA+AG genotypes were more associated with the plaque type.

The majority of Saudi psoriasis cases were carriers of the insertion allele while about 15% were homozygous for the deletion allele of NF-κB1 gene which was found to result in relatively decreased NF-κB1 transcript levels and hence decreased p50/p105 NF-κB1 protein production. In this respect, we speculate that the balance between the expressions of the 3 genes might be responsible for the increased expression of NF-κB in the psoriatic lesions as was previously reported. Similarly, in a study conducted among Chinese psoriatic patients Li et al., reported only a marginal association between the NF-κB1-94 ins/del ATTG Ins/Ins genotype and the increased risk of psoriasis vulgaris in the cases-subgroups of onset age <or=40, PASI >20, male patients and sporadic (non-familial) patients. On the other hand, Butt et al., did not observe an association between NF-κB1-94 ins/del ATTG promoter polymorphism in patient with psoriatic arthritis (PsA) from Newfoundland. Comparing the distribution of NF-κB1 -94del/ins ATTG polymorphisms among Saudi controls to the controls in other studies, we could observe some variations related to ethnicity. The frequencies of the del/del, del/ins, and ins/ins genotypes of the NF-κB1 among Saudi controls were 17.5%, 41.3% and 41.2% respectively that was somewhat similar to the frequencies among German (15.6%, 45.9%, and 38.4%, respectively); but different to the frequencies among Han Chinese (18.5%, 51.9%, and 29.6%, respectively) and other Chines (17%, 58%, and 24%, respectively). Salim et al. have also recently reported a negative association of the allelic and genotype distribution of the NF-κB promoter polymorphism with the susceptibility, clinical pattern and laboratory features of systemic sclerosis among Brazilians. However,
several studies have reported that the NF-κB1 and NF-κB1A polymorphisms is related to the development of inflammatory and other diseases including ulcerative colitis, Graves' disease, and diabetes mellitus, and susceptibility to tumors including melanoma, bladder cancer and colorectal cancer in different ethnic groups. (22, 50-54) Similar to our finding, Li et al., did not detect an association of the SUMO4 rs237025 A>G polymorphism with the susceptibility and clinical pattern of psoriasis vulgaris among Chinese patients. (45) In contrast, another study has confirmed the association between this SNP of SUMO4 polymorphism and psoriatic patients in USA. (41) A meta-analysis study has demonstrated the association of SUMO4 M55V polymorphism with other autoimmune and inflammatory diseases, especially in Asian population. (55) Researchers have also reported a weak association of SUMO4 with type 1 diabetes that was noted in the Caucasian populations, (56-58) while a strong association was found with type1 diabetes in Asian populations. (30, 59) An association was also reported between the SUMO4 polymorphism with RA and autoimmune thyroid disease (Graves’ disease and Hashimoto disease) in the Japanese population; (31) and with BD in Chinese patients; (34) although this association was negative with SLE, (33) RA, (60) BD in Korean patients (35) and with Graves’ disease (61) in English patients. Hou et al., have also reported a non-association of the SUMO4 polymorphisms with Vogt-Koyanagi-Harada (VKH) syndrome among Chinese Han population. (36)

Table 2. Demographic and clinical data of studied cases of psoriasis related to their genotypic polymorphisms

<table>
<thead>
<tr>
<th></th>
<th>NF-κB1-94 ins/del</th>
<th>NF-κB1A A&gt;G</th>
<th>SUMO4 G&gt;A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II %</td>
<td>ID +DD %</td>
<td>P</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=45)</td>
<td>38.6</td>
<td>61.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Female (n=38)</td>
<td>38.8</td>
<td>61.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque (n=70)</td>
<td>32.9</td>
<td>67.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Guttate (n=9)</td>
<td>44.4</td>
<td>55.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Family History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=68)</td>
<td>36.2</td>
<td>63.8</td>
<td>0.97</td>
</tr>
<tr>
<td>Positive (n=14)</td>
<td>35.7</td>
<td>64.3</td>
<td>0.70</td>
</tr>
<tr>
<td>Consanguinity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=64)</td>
<td>32.0</td>
<td>68.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Positive (n=19)</td>
<td>36.8</td>
<td>63.2</td>
<td>10.5</td>
</tr>
<tr>
<td>PASI Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>5.9±5.1</td>
<td>4.4±3.1</td>
<td>0.27</td>
</tr>
<tr>
<td>Age of onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>23.6±11.9</td>
<td>24.0±10.9</td>
<td>0.87</td>
</tr>
</tbody>
</table>
have an association with the clinical type and severity of the disease.

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**References:**


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