Assessment of Genetic Variations Associated With Susceptibility to Psoriasis Among Iraqi Population

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Abstract

Background: Psoriasis is an immune–mediated, chronic hyperproliferative disease of the skin. Genetic association studies have identified multiple psoriasis risk loci; however, the frequency of variations at these loci differs quite strikingly among the populations, suggesting heterogeneity in the genetic susceptibility to psoriasis.

Aim of the study: to assess the possible association of selected genetic variants with psoriasis among Iraqis.

Methodology: In this case-control study, 50 psoriasis patients and 40 control subjects were enrolled. Peripheral blood was obtained from all patients and control subject and used for extraction of genomic DNA. Amplification Refractory Mutation System (ARMS-PCR) was used to detect 3 SNPs in IL-20RA (Rs1184860, Rs1167846 and Rs1167849), whereas conventional PCR was used to assess deletions in late cornified envelope 3B and late certified envelope 3C (LCE3B and LCE3C) genes.

Results and Discussion: Nine haplotypes were identified by SNPs at IL-20RA gene; the haplotype TTA (Rs1184860, Rs1167846 and Rs1167849, respectively) was associated with psoriasis 19(38%), whereas the CTA haplotype had a protective effect 12(30%). The frequencies of LCE3B deletion in both patients and control groups were 14(28%) and 20(50%) respectively, while the frequencies of LCE3C deletion were 16(32%) and 20(50%) among patients and control respectively. Statistical analysis revealed a significant association between LCE3B deletion and susceptibility to the psoriasis (P < 0.05). The frequencies of homozygous deletion (LCE3B_LCE3C del) in both patients and control groups were 13(26%) and 9(22.5%) respectively. The statistical analysis had revealed a non significant (P > 0.05) association between LCE3C deletion and homozygous deletion (LCE3B_LCE3C del) with susceptibility to psoriasis.

Conclusion: The haplotype TTA of IL-20RA gene has a role in the susceptibility to the psoriasis among Iraqi population. In addition, no association was found between LCE3B_LCE3C deletion and psoriasis in Iraqi population.

Keywords: psoriasis, IL-20RA, LCE3C, LCE3B, SNPs, deletion

Introduction

Psoriasis is an immune–mediated chronic hyperproliferative disease of the skin, with a worldwide prevalence of approximately 1 to 3% (¹). It is a complex, multifactorial disease that appears to be influenced by genetic, environmental factors and immune-mediated components (²). A number of studies have supported the finding that genetic predisposition has a critical role in the development of psoriasis (³). By using linkage analysis and genome-wide association studies (GWAS), several loci have been identified as risk factors for the development of psoriasis (⁴,⁵). There is increasing evidence to suggest that cytokine interleukin-20 (IL-
20) has a role in the pathogenesis of cutaneous inflammation in psoriasis (6-8). Indeed, IL-20 has important functions in skin (7). Moreover, IL-20 has been implicated to play an important role in several autoimmune diseases (9). Interleukin 20 gene and protein expression was elevated in lesional psoriatic skin compared with normal and non lesional skin (10,11). Furthermore, several susceptibility loci for psoriasis have been found by genetic analyses in multiply affected families (12). Several studies have focused on the deletion of two genes of the late cornified envelope (LCE) gene family, LCE3B and LCE3C. The LCE gene cluster, which is composed of six groups (LCE 1-6, with a total of 18 members) is a part of the epidermal differentiation complex. Its deletion has been strongly linked with psoriasis (13). Several studies have focused on the deletion of two genes of the late cornified envelope (LCE) gene family, LCE3B and LCE3C. Deletion of LCE3B and LCE3C genes are present in (60-70 %) of the general population and shown to be at higher frequency in European psoriasis patients (14).The exact function of LCE3B and LCE3C genes is not known but they are induced after minor skin trauma such as tape stripping (15).

This study aimed to investigate the contributions of SNPs at IL-20RA and deletions of LCE3B and LCE3C genes in the susceptibility to the psoriasis.

Materials and Methods

Patients
This study included 90 subjects (50 psoriasis patients and 40 control subjects) with age range from 4 to 60 years old. All patients and control subjects were attendants of Dermatology Out-Patient Clinic in Hospital of Imam AL-Hussein Medical City in Kerbala Province during the period from January, 2013- June, 2013.

Blood samples:
One ml of venous blood was collected from each participant. Blood collected in EDTA, heparin and citrate anticoagulant tubes.

DNA extraction from fresh blood samples
The DNA was directly extracted from 300μl of fresh non coagulated whole blood using the genomic DNA Mini Kit (Blood/ Cultured Cell) Fresh Blood Protocol/geneaid company (Korea). The DNA extracts were stored at -20°C until be used.

Mutations and SNPs:
To detect (rs1184860 T/C, rs1167846 C/T and rs1167849 G/A) polymorphisms in the IL-20RA gene, located on chromosome 6q22.33-23.1, and detect the LCE3B and LCE3C genes mutations (these genes encode members of late cornified envelope (LCE), and are in the region that contains the PSORS4 locus on chromosome 1q21),the primer sequences, product size,PCR conditions and references as listed in table-1.

Results

IL-20RA genetic polymorphism
Analyzing the distribution of the 3 SNPs all together (Rs1184860, Rs1167846 and Rs1167849) revealed several important patterns and haplotypes. The TTA haplotypes was the highest percentage among the psoriasis cases (found in 19 out of 50, 38%), whereas it was found only in 6 out of 40 control subjects, (15%). In contrast, the CTA was the high percentage haplotypes among the control subjects (12 out of 40, 30%) while it was only found in 7 (14%) of the psoriatic patients. Thus, it seems that the haplotype TTA is more linked to the susceptibility to psoriasis and haplotype CTA is more linked to the resistance to this disease as estimated control. Furthermore, the T-G was double folded detected 10(20%) among the psoriasis cases than in control subjects 4(10%).
Table 1. primers sequences, product size and PCR condition for detecting the IL-20RA polymorphism and LCE3B and LCE3C genes mutation.

<table>
<thead>
<tr>
<th>Polymorphism Location Allele</th>
<th>Primer Sequence</th>
<th>Size</th>
<th>PCR condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1184860 T/C</td>
<td>Forward inner primer (C allele): TTTTATGTAGTAAAGAATGACAGCCG</td>
<td>289 bp</td>
<td>95°C 5 min 1x</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>Reverse inner primer (T allele): TTTTGGGTTATGTTTATGGCATCTTCAAA</td>
<td>193 bp</td>
<td>60°C 1 min 40x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forward outer primer: TTTATAGTAGAGATGGGTTITTCATG</td>
<td>155 bp</td>
<td>72°C 1 min 72°C 7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse outer primer: AAGATCTTGTTGTTTCAAGCATAGCA</td>
<td>1 bp</td>
<td>72°C 7 min 1x</td>
<td></td>
</tr>
<tr>
<td>rs1167846 C/T</td>
<td>Forward inner primer (C allele): CAGTCATCCAATCTATTTTATTGGGGGC</td>
<td>376 bp</td>
<td>95°C 5 min 1x</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>Reverse inner primer (T allele): AGAGGAGACTACATCATATTTATTGGGGGC</td>
<td>244 bp</td>
<td>94°C 1 min 60°C 1 min 40x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forward outer primer: TACTCTGGTTTATGTTTAGTTCCGAGA</td>
<td>188 bp</td>
<td>72°C 1 min 72°C 7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse outer primer: CCACCTGATTCGATATGCTCATGTT</td>
<td>1 bp</td>
<td>72°C 1 min 1x</td>
<td></td>
</tr>
<tr>
<td>rs1167849 G/A</td>
<td>Forward inner primer (A allele): CTTTGGGTAATGGGAAATGCTCCAAAA</td>
<td>356 bp</td>
<td>95°C 5 min 1x</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>Reverse inner primer (G allele): TATAACCTTTCTCCACACACTTCC</td>
<td>236 bp</td>
<td>94°C 1 min 60°C 1 min 40x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forward outer primer: AGAAAAGCTCCGGAATTATTCGCTCAG</td>
<td>178 bp</td>
<td>72°C 1 min 72°C 7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse outer primer: AACTATGAACAGTTCCACAGGAAGGC</td>
<td></td>
<td>72°C 7 min 1x</td>
<td></td>
</tr>
<tr>
<td>LCE3B</td>
<td>GGGCTTCATAAAAACCATTTTGAGAG (forward)</td>
<td>422 bp</td>
<td>94°C 4 min 1x</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>TTTTCTCTAAAGCTCGTCTGCTCA (reverse)</td>
<td></td>
<td>94°C 30 sec 63°C 30 sec 30x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TCTGGAAAAGCATGACATCAGG (forward)</td>
<td>448 bp</td>
<td>94°C 4 min 1x</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>TCTGGAAAAGCATGACATCAGG (reverse)</td>
<td></td>
<td>94°C 30 sec 62°C 30 sec 30x</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Haplotypes of psoriasis patients and control associated with IL-20RA polymorphism.

<table>
<thead>
<tr>
<th>IL-20RA haplotypes</th>
<th>Psoriatic patients no. (50)</th>
<th>Control no. (40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive result</td>
<td>Positive result</td>
</tr>
<tr>
<td>TTG</td>
<td>2(4%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>CTG</td>
<td>27(54%)</td>
<td>19(47.5%)</td>
</tr>
<tr>
<td>TTA</td>
<td>19(38%)</td>
<td>6(15%)</td>
</tr>
<tr>
<td>CTA</td>
<td>7(14%)</td>
<td>12(30%)</td>
</tr>
<tr>
<td>T-G</td>
<td>10(20%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>TGA</td>
<td>6(12%)</td>
<td>5(12.5%)</td>
</tr>
<tr>
<td>CGA</td>
<td>2(4%)</td>
<td>3(7.5%)</td>
</tr>
<tr>
<td>TC-GA</td>
<td>3(6%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>C-A</td>
<td>0</td>
<td>1(2.5%)</td>
</tr>
</tbody>
</table>
In addition, CTG haplotype was also found in psoriasis cases in 27(54%) while it was found in 19(47.5%) of control. Haplotype C-A was found in 1(2.5%) of control subjects but not found in psoriasis cases as shown in table-2.

In the present study investigated the distribution of three SNPs in the IL20RA gene in 50 psoriasis patients and 40 age matched control subjects. Interestingly, association analysis of haplotypes revealed association of the TTA haplotype with psoriasis (38%), whereas carriage of the CTA haplotype seemed to have a protective effect (30%) as it was shown to have more association with the control group. Therefore, this study supports the evidences that suggest the role of polymorphisms in the IL20RA gene in the development of psoriasis.

**LCE3B and LCE3C genes mutation**

The results of LCE3B_LCE3C genes deletions in patients with psoriasis, and in normal control subjects are presented in table (3). LCE3B was deleted in 14(28%) of the cases and in 20(50%) of the control subjects whereas LCE3C was deleted in 16(32%) of the cases and 20(50%) of the control subjects. Homozygous deletion (LCE3B_LCE3C del) was reported in 13(26%) out of 50 of the cases and 9(22.5%) out of 40 of the control subjects. The difference between patients and control in term of LCE3B deletion was significant (P < 0.05), however LCE3C deletion was insignificantly different between patients and control (P > 0.05). The incidence of homozygous LCE3B_LCE3C deletion was higher among cases, however this difference was statistically non significant (P > 0.05), Chi square (X²), were used to determine the significant level of the difference in genotypes.

<table>
<thead>
<tr>
<th>Study groups (n.)</th>
<th>LCE3B gene</th>
<th>LCE3B_LCE3C genes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>deletion</td>
<td>No-deletion</td>
<td>deletion</td>
</tr>
<tr>
<td>Psoriasis patients</td>
<td>14(28%)</td>
<td>36(72%)</td>
<td>16(32%)</td>
</tr>
<tr>
<td>n = 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control n=40</td>
<td>20(50%)</td>
<td>20(50%)</td>
<td>20(50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Interleukin-20 is a member of the IL-19 subfamily of cytokines (18,19). This subfamily cytokines are important regulators of epidermal keratinocyte biology and are important in the immunopathology of psoriasis (20-22) and overexpression several members of this subfamily such as IL-19, IL-20 and IL-24 was detected in lesional skin of psoriasis patients in comparison to healthy skin (23,24).

Previous studies have indicated the importance of IL-20 in the manifestation of psoriasis (25,10). In last studies the hypothesis is tested, that genetic variants of IL-20-RI influence susceptibility to psoriasis (18). Therefore, the mechanistic explanation of the association of certain SNPs with the development of psoriasis is based on the effect of the presence of those SNPs on the expression of IL20R.

It's noteworthy that the type of haplotypes associated with psoriasis/ control are different from that reported in previous study. Kingo et al found a significant association of the IL20RA (CCG) haplotype with psoriasis (7.6%) in compere with (2.6%) in control, whereas carriage of the IL20RA (TTC) haplotype suggested to have a protective effect (4.4%) in control in compere with (0.9%) in psoriasis patients (16). The discrepancy in the results may be due to the difference in...
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The high frequency of the deletion worldwide suggests the existence of some redundancy in the function of LCE genes in this cluster. It is possible that other genes fulfill the function of LCE3C and LCE3B, although imperfectly, contributing to the abnormal differentiation and epidermal hyperproliferation characteristic of psoriatic lesions. Thus, when other susceptibility components are not present, the deletion is insufficient to produce the abnormal phenotype but when several susceptibility components concur, the LCE3B_LCE3C deletion could lead to disease development.

In Chinese population, LCE3B_LCE3C-del was found to play a role in susceptibility to psoriasis, especially in patients with early-onset psoriasis and/or with a positive family history. These findings suggest that the LCE gene cluster is an important candidate gene in the pathogenesis of psoriasis. The absence of LCE3C and LCE3B may trigger skin barrier function in epithelia and then induce expression of the other late cornified envelope LCE genes in the cluster. This then results in abnormal differentiation and hyperproliferation of epidermis. LCE3B and LCE3C genes are also induced after minor skin trauma and deletion of these proteins leads to incomplete barrier repair after minor trauma which in turn causes penetration of various antigens and induction of inflammatory response. Statistical analysis demonstrated that the deletion was significantly associated with the risk of psoriasis in over 2,800 samples from Spain, the Netherlands, Italy and the United States.

The discrepancy between these studies and this study could be partly attributed to two factors; first is related to ethnicity, as other studies conducted on ethnic groups differ from Iraqis, secondly may be due to the small volume of samples in this study. Wiwanitkit (2010) reported that there is no relationship between LCE3C_LCE3B-del genotype and psoriasis. Another study by Ammaret al. (2014) failed to detect any evidence of association between LCE3C_LCE3B-del and psoriasis in Tunisian population. These studies agree with this study that suggest no association between LCE3B_LCE3C deletion and psoriasis in Iraqi population.

Conclusions

1- The vast majority of single nucleotide polymorphism in the Interleukin-20RA gene have a clinical importance regarding psoriasis.
2- The TTA haplotype of Interleukin-20RA gene has association as a predisposing factor for psoriasis, while CTA haplotype has association as a protective factor for psoriasis.
3- There is an interaction among the single nucleotide polymorphism of Interleukin-20 gene occurring in the same individual. This interaction can result in increasing the severity of inflammatory response.
4- This study confirmed an association between the Late cornified envelope 3B gene (LCE3B) deletion mutation and susceptibility to psoriasis.

Recommendations

1. Conducting massive studies on psoriasis single nucleotide polymorphism that characterized Iraqi populations.
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2. Conducting further studies to confirm the genetic association and to investigate the functional relevance of Interleukin-20RA haplotypes in psoriasis.

3. Investigating the role of polymorphisms in other genes that may affect the immune response to psoriasis.

4. Patients with TTA haplotype of Interleukin-20RA are at risk for psoriasis. Thus these patients should subject periodically for clinical investigation for this disease.

References


