Polymorphism in the IL-1 Receptor Antagonist Gene in Vitiligo

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Background: The severity of several chronic inflammatory diseases was reported to be associated with polymorphism of the IL-1 receptor antagonist gene (IL-1rn).
Objective: This study was performed to study the polymorphism of the IL-1rn in vitiligo and in the normal Korean population.
Methods: Thirty one cases of vitiligo and seventy nine normal Koreans as control were studied for the polymorphism of IL-1rn.
Results: The frequency of allele 2 of the IL-1rn in 31 patients with vitiligo was compared with that of the 79 healthy controls. The frequency of allele 2 was 1.6% in vitiligo patients and 3.8% in the normal controls.
Conclusion: There was no significant difference in the frequency of allele 2 between the vitiligo patients and normal controls. (Ann Dermatol 7(4)299~302, 1995)

Key Words: IL-1 receptor antagonist, IL-1receptor antagonist gene, Polymorphism, Vitiligo

Vitiligo is a common pigmentary disorder affecting at least 1% of the world's population. Although several hypotheses have been proposed, the exact pathogenesis of melanocyte damage is not clear. The frequent association of vitiligo with hyperthyroidism, pernicious anemia, diabetes mellitus, and Addison's disease raised the possibility that vitiligo might be an antibody associated with an autoimmune disease. Mild inflammatory lymphocytic infiltrates, particularly at the margin of enlarging lesions, have been observed. It appears that vitiligo is likely to be a polygenic disease with different loci contributing to susceptibility and severity. Environmental factors such as trauma, ultraviolet lights have also been implicated in the initiation of vitiligo. Because interleukin-1(IL-1), that plays a central role in the regulation of immune and inflammatory responses, can be released by ultraviolet radiation and trauma, the proinflammatory cytokine IL-1 is a strong candidate for involvement in melanocyte destruction.

Interleukin-1 receptor antagonist(IL-1ra), a competitive antagonist for the IL-1 receptor, regulates the action of IL-1 for the immune and inflammatory responses. The gene for IL-1ra(IL-1rn) is located on the long arm of chromosome(2q14-q21) on a 430kb stretch of DNA that also contains the genes for IL-1α and IL-1β. The IL-1 ra is a 23-25kDa glycoprotein structurally related to IL-1α and IL-1β. It binds to both types of IL-1 receptor but fails to initiate intracellular signaling and acts as a competitive antagonist of IL-1. Tarlow et al have described a variable number tandem repeat polymorphism in intron 2 of the IL-1ra gene. Five alleles of the system were identified corresponding to 2,3,4,5 and 6 copies of an 86-bp repeat sequence(Fig. 1). It was reported that allele 2 of IL-1rn is associated with chronic inflammation in several different diseases, including psoriasis,
lichen sclerosis and inflammatory bowel disease, although Rosbotham et al exclude IL-1Rn from a major genetic determinant for psoriasis by two point linkage analysis. In view of inflammatory features of vitiligo such as lymphocytic inflammatory infiltrate around blood vessels and adnexal structure, we decided to test the genetic association of vitiligo with the IL-1Rn polymorphism by comparing the allele frequencies in vitiligo patients with those in a large control population of healthy individuals.

**MATERIALS AND METHODS**

**DNA and oligonucleotide preparation**

Blood was collected from 79 unrelated, healthy Korean individuals and from 31 vitiligo patients attending the dermatology outpatient clinic at the Seoul National University Hospital. Extraction of DNA was performed directly from dried blood spots. The oligonucleotide primers used for polymerase chain reaction (PCR) were prepared according to the Tarlow et al, and the primer sequences were 5′CTCAGCACAACATCTCCTAT3′ and 5′TCCTGOTTCTGCAGGTA3′.

**Polymorphism typing**

The method was based on a PCR as previously described. PCR conditions comprised 30 cycles of 94°C for 20 seconds, 60°C for 1 minute and 70°C for 1 minute. The alleles were identified by size separation of PCR products on a 6% polyacrylamide gel stained with silver.

**Statistical analysis**

The occurrence of each allele in two groups was expressed as a percentage of the total number of alleles present to give a frequency. Chi-squared test was performed on frequencies to determine p values.

**RESULTS**

The five alleles of the IL-1Rn gene polymorphism corresponded to 2 to 6 copies respectively of the 86-bp repeat (Fig. 1). Allele 4 and 5, corresponding to three and six copies of the 86-bp repeat respectively, were not found in either population described here. Three alleles were observed (Fig. 2), all corresponding to 2, 4, and 5 copies of the 86-bp sequence. The size and frequencies of these alleles are shown in Table 1.
Table 1. IL-1 m Allele frequencies and sizes in controls and vitiligo patients

<table>
<thead>
<tr>
<th></th>
<th>Number of repeats</th>
<th>Size of allele</th>
<th>Frequencies (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Controls (n=79)</td>
</tr>
<tr>
<td>A1</td>
<td>4</td>
<td>410bp</td>
<td>94.9</td>
</tr>
<tr>
<td>A2</td>
<td>2</td>
<td>240bp</td>
<td>3.8</td>
</tr>
<tr>
<td>A3</td>
<td>5</td>
<td>500bp</td>
<td>1.3</td>
</tr>
<tr>
<td>A4</td>
<td>3</td>
<td>325bp</td>
<td>0</td>
</tr>
<tr>
<td>A5</td>
<td>6</td>
<td>595bp</td>
<td>0</td>
</tr>
</tbody>
</table>

The allele frequencies in 79 individuals from the general population were compared with the vitiligo patients. The frequency of the 2 repeat allele is 3.8% in normal controls and 1.6% in that of vitiligo patients. However, there was no significant differences between the groups. (chi-squared p value >0.05)

DISCUSSIONS

Interleukin-1(IL-1), a strong candidate for involvement in inflammatory disease such as alopecia areata and rheumatoid arthritis, is one of the most potent proinflammatory mediators. Its actions are regulated by a structurally related antiinflammatory cytokine known as the interleukin-1 receptor antagonist(IL-1ra). The IL-1ra is structurally similar to IL-1α and IL-1β and competes with these molecules for occupancy of IL-1 cell surface receptor. Since it does not induce signal transduction, it acts as a competitive inhibitor. It may be important in many IL-1 mediated diseases, by acting as an endogenous regulator of inflammation.

Tarlow et al investigated the polymorphism in intron 2 of the IL-1ra gene and identified polymorphism caused by the variable number of an 86-bp sequence. Recently it was reported that there was a significant association between allele 2 of the polymorphism of the IL-1rn gene and several chronic inflammatory diseases. The number of repeats may be of functional significance as the sequence contains three potential protein binding sites; an α-interferon silencer A, a β-interferon silencer B and an acute phase response element. The α-interferon silencer A is contained in the viral response element and acts as a repressor when four tandem copies are positioned between an enhancer and a promoter. Tetramers of these sequences can mediate inducibility by virus; however, further characterization is in progress. 

A large number of studies have demonstrated the ability of IL-1ra to block the ability of IL-1 both in vivo and in vitro. IL-1ra blocks IL-1 induced changes like increased circulating IL-6, increased corticosterone in mice, and neutrophilia in mice in vivo, and lymphocyte proliferation, PGE synthesis in fibroblasts and synovial cells, and synthesis of cytokines from monocytes in vitro. Currently recombinant IL-1ra is being tested in therapeutic trials for the treatment of chronic myelogenous leukemia, rheumatoid arthritis and septic shock.

Considering the inflammatory features of vitiligo similar to other inflammatory skin diseases as mentioned above, we aimed at discovering the differences of the IL-1rn polymorphism in vitiligo patients, and comparing the frequency of allele 2 of the IL-1rn polymorphism with that of normal populations. We could find no differences in the frequency of allele 2 between vitiligo and normal population in this study. However, it needs further study using a large number of people as a study group and correlation with vitiligo classification according to type and severity.

REFERENCES

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