EXTENDED REPORTS

Association of oestrogen receptor gene polymorphisms with age at onset of rheumatoid arthritis

Toshio Ushiyama, Kanji Mori, Koji Inoue, Jie Huang, Junichi Nishioka, Sinsuke Hukuda

Abstract

Objective—In view of the possible role of oestrogens in the pathogenesis of rheumatoid arthritis (RA), this study investigated the association between oestrogen receptor (OR) gene polymorphisms and RA.

Methods—Pvu II and Xba I restriction fragment length polymorphisms of the OR gene were analysed in 70 male and 240 female patients with RA, and in 300 male and 350 female controls. The absence or presence of restriction sites were represented as P, p (Pvu II) or X, x (Xba I). The distribution of OR genotypes was compared between the RA and control subjects by sex. RA patients were divided into subgroups according to their OR genotypes, then the age at onset, seropositivity, and rheumatoid nodule positivity were compared between the subgroups.

Results—The OR genotype frequency of distribution did not have significant differences between the male RA and male controls nor between the female RA and female controls. In women with RA, there was a significant difference of age at onset between the subgroups (uncorrected p=0.047, corrected p=0.94). Female patients with the OR genotype PPxx (homozygote of Px) tended to have developed RA at a younger age, whereas those with PPXx and ppxx (lack of Px haplotype) developed RA at an older age. In men with RA, there was no association between the OR genotype and age at onset. In seropositivity and rheumatoid nodule positivity, there was no significant difference between subgroups for either sex.

Conclusions—Some variants of the OR gene are related to the onset of RA in women in certain age periods, suggesting the role of the interaction between the OR gene and serum concentrations of oestrogens at the onset of the disease. (Ann Rheum Dis 1999;58:7–10)

Rheumatoid arthritis (RA) is an autoimmune disease that affects women more often than men, and the severity of their symptoms is influenced by pregnancy, delivery, and menstrual cycles. The onset of symptoms is often associated with menopausal transition. With these clinical observations, the role of sex hormones such as oestrogens in the pathogenesis of RA has been discussed.

On the other hand, a genetic factor in RA is also well recognised. It has been reported that 15.4% of the monozygotic twins were disease concordant for RA. Recently, the existence of an oestrogen receptor (OR) gene polymorphism has been made clear, and its association to some variant OR genotypes with breast cancer, hypertension, osteoporosis, and generalised osteoarthritis has been reported. Assuming that individual reaction to oestrogens is genetically determined by OR gene polymorphisms, we hypothesised that some variant of OR genotypes would be related to the onset of RA in some age periods characterised by hormonal status. In consideration of the immunomodulatory effects of oestrogens, we further hypothesised that the OR genotypes might be related to the severity variables such as seropositivity and rheumatoid nodule positivity. To ascertain these hypotheses, we investigated the relation of OR gene polymorphisms with clinical variables of RA.

Methods

SUBJECTS

A total of 310 patients with RA who visited our outpatient clinic between October 1996 and March 1997 were studied. They all fulfilled the American College of Rheumatology 1987 criteria. Seventy were men and 240 were women. Two women with disease onset under age 16 were included in this study. The clinical features of these patients such as age at onset, seropositivity, and the presence of nodules were recorded. Determination of the age at onset was based on the patient’s recollection. Periph-

Table 1 Characteristics of RA

<table>
<thead>
<tr>
<th></th>
<th>Male RA (n=70)</th>
<th>Female RA (n=240)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>59.6 (22–82)</td>
<td>56.6 (25–85)</td>
</tr>
<tr>
<td>Age at onset (y)</td>
<td>50.3 (18–82)</td>
<td>45.2 (11–81)*</td>
</tr>
<tr>
<td>Seropositivity (%)</td>
<td>92.9</td>
<td>92.5</td>
</tr>
<tr>
<td>Rheumatoid nodule positivity (%)</td>
<td>12.9</td>
<td>13.3</td>
</tr>
</tbody>
</table>

* p = 0.007.
Table 2 Frequency of OR genotypes and relative risks for developing RA

<table>
<thead>
<tr>
<th>OR genotypes</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA % (n)</td>
<td>Control % (n)</td>
</tr>
<tr>
<td>PPXX</td>
<td>4.3 (3)</td>
<td>3.7 (11)</td>
</tr>
<tr>
<td>PPXx</td>
<td>4.3 (3)</td>
<td>8.3 (25)</td>
</tr>
<tr>
<td>PpXx</td>
<td>11.4 (8)</td>
<td>5.7 (17)</td>
</tr>
<tr>
<td>PpXx</td>
<td>15.7 (11)</td>
<td>23.0 (69)</td>
</tr>
<tr>
<td>PpXx</td>
<td>37.2 (26)</td>
<td>26.3 (79)</td>
</tr>
<tr>
<td>PpXX</td>
<td>11.4 (8)</td>
<td>11.1 (33)</td>
</tr>
<tr>
<td>PPXX</td>
<td>4.3 (3)</td>
<td>3.7 (11)</td>
</tr>
</tbody>
</table>

5% CI: 95% confidence intervals.

Table 3 Age at onset, seropositivity, and rheumatoid nodule positivity by OR genotypes

<table>
<thead>
<tr>
<th>OR genotypes</th>
<th>n</th>
<th>Age at onset (y)</th>
<th>RF (+) % (n)</th>
<th>RN (+) % (n)</th>
<th>p value</th>
<th>n</th>
<th>Age at onset (y)</th>
<th>RF (+) % (n)</th>
<th>RN (+) % (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPXX</td>
<td>3</td>
<td>50.0</td>
<td>66.7 (2)</td>
<td>0.0 (0)</td>
<td>0.94</td>
<td>7</td>
<td>53.1</td>
<td>85.7 (6)</td>
<td>0.0 (0)</td>
<td>0.94</td>
</tr>
<tr>
<td>PPXx</td>
<td>3</td>
<td>45.0</td>
<td>100.0 (3)</td>
<td>33.3 (1)</td>
<td>0.15</td>
<td>21</td>
<td>42.7</td>
<td>100.0 (21)</td>
<td>4.8 (1)</td>
<td>0.15</td>
</tr>
<tr>
<td>PpXx</td>
<td>8</td>
<td>52.8</td>
<td>100.0 (8)</td>
<td>25.0 (2)</td>
<td>0.23</td>
<td>15</td>
<td>40.5</td>
<td>80.0 (12)</td>
<td>6.7 (1)</td>
<td>0.23</td>
</tr>
<tr>
<td>PpXX</td>
<td>11</td>
<td>49.8</td>
<td>100.0 (11)</td>
<td>18.2 (2)</td>
<td>0.17</td>
<td>53</td>
<td>42.3</td>
<td>92.5 (49)</td>
<td>7.5 (4)</td>
<td>0.17</td>
</tr>
<tr>
<td>PpXX</td>
<td>26</td>
<td>51.7</td>
<td>88.5 (23)</td>
<td>11.5 (3)</td>
<td>0.13</td>
<td>60</td>
<td>45.6</td>
<td>91.7 (55)</td>
<td>20.0 (12)</td>
<td>0.13</td>
</tr>
<tr>
<td>PpXX</td>
<td>3</td>
<td>48.5</td>
<td>94.7 (18)</td>
<td>5.3 (1)</td>
<td>0.33</td>
<td>84</td>
<td>47.6</td>
<td>94.0 (79)</td>
<td>16.7 (14)</td>
<td>0.33</td>
</tr>
</tbody>
</table>
p value 0.94 0.15 0.13 0.047 0.98 0.36

RF: rheumatoid factor, RN: rheumatoid nodule.

The difference in the continuous variable between two groups was evaluated by a Student t test, while the differences between more than three groups was evaluated by analysis of variance (ANOVA). A χ² test was also used when appropriate. The software used for the analysis was SPSS (SPSS Inc, Chicago, USA).

OR GENE POLYMORPHISM ANALYSIS

An analysis of OR gene restriction fragment length polymorphisms (RFLPs) in the intron 1 was performed according to the methods of the previous report. Briefly, 100 ng of genomic DNA, extracted from peripheral blood, was amplified by a polymerase chain reaction (PCR) using 2.5 µM of specific oligonucleotide primers, 0.2 µM each of dNTP (TAKARA SHUZO, Otsu, Japan) and 0.2 µM each of specific oligonucleotide primers in the same PCR conditions.

The product of PCR was digested with a restriction endonuclease, Pvu II or Xba I (TAKARA SHUZO) and electrophoresed on 1.0% agarose gel. The RFLPs also were represented by P and p (Pvu II), and X and x (Xba I), with capital and small letters signifying the absence or presence of restriction sites, respectively.

STATISTICAL ANALYSIS

The frequency of distribution of each genotype was compared between the RA group and controls by sex. An odds ratio and 95% confidence intervals (CI) were calculated with respect to the presence of the reference OR genotype.

Table 4 Age at onset, seropositivity, and rheumatoid nodule positivity by Px haplotypes

<table>
<thead>
<tr>
<th>Px haplotypes</th>
<th>Male RA (n=59)</th>
<th>Female RA (n=187)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Age at onset (y)</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>52.8</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>51.0</td>
</tr>
<tr>
<td>00</td>
<td>22</td>
<td>48.7</td>
</tr>
</tbody>
</table>
p value 0.75 0.15 0.13 0.056 0.88 0.69

Px haplotype 11 (Px/Px): Ppxx; 10 (Px-): PPXx, Ppxx; 00 (-/-): PPXX, ppXX.
RA patients and male controls nor between the female RA patients and female controls in the frequency of any genotype.

CLINICAL VARIABLES OF RA IN EACH OR GENOTYPE

Patients with RA were divided into six groups according to OR genotypes by sex. Age at onset, seropositivity, and rheumatoid nodule positivity were compared between the groups (table 3). There was no significant difference between the groups in male RA age at onset, however there was in the female RA groups (uncorrected p=0.047 using ANOVA, corrected p=0.94). The mean age at onset for the patients with OR genotype PPxx was 40.5, PpXx 42.3, PPXx 42.7, Ppxx 45.6, ppxx 47.6, and PPXX 53.1, respectively. Post hoc test by Scheffe’s method, however, showed no significant difference of mean age at onset between groups with a significant level of 0.05. Regarding seropositivity and rheumatoid nodule positivity, there was no significant difference found between groups of either sex by the $\chi^2$ test.

In a separate analysis, patients with RA were grouped into three groups according to the Px allelic haplotype, in which the genotype PPxx is a homozygote (Px haplotype 11), PPXx and Ppxx are heterozygotes (Px haplotype 10), and PPXX and ppxx would be characterised by the lack of the Px haplotype (Px haplotype 00).10 Because the haplotype of PpXx is unclear, patients with this genotype were excluded from this analysis. Age at onset, seropositivity, and rheumatoid nodule positivity were then compared between these groups (table 4). There was no significant difference in any of these clinical parameters for both sexes. However, in female RA, three groups tended to have a different age at onset (uncorrected p=0.056 using ANOVA, corrected p=0.11). The mean ages at disease onset in Px haplotype 11, 10, and 00, were 40.5, 44.8, and 48.0, respectively.

Discussion

This study suggests that some of the OR gene polymorphisms were related to the age at onset in women with RA. There was a significant difference between six genotype groups in female RA age at onset. Female patients with Px haplotype 11 tended to have developed the disease at a younger age, those with Px haplotype 10 at an intermediate age and those with Px haplotype 00 at a relatively older age, suggesting that the Px allelic haplotype may play some part in the onset of the disease in women.

The serum oestrogen concentration in women is highest during childbearing age, and then rapidly decreases after menopause. Although we did not examine menopausal status at disease onset, the peak age at onset of female RA, having Px haplotype 11, was about the premenopausal period, whereas that of women having Px haplotype 00 about the perimenopausal or postmenopausal period. Given that variants of OR gene polymorphisms would determine the reaction to hormonal stimuli, the coexistence of such a genetic predisposition and exposure to a specific hormonal environment might be related to the onset of RA.

The characteristic clinical features of older age onset RA have been described by many authors.14 15 An equal distribution of the disease between men and women, a tendency towards large joint involvement, an increased erythrocyte sedimentation rate, and less seropositivity have been mentioned as characteristic manifestations of older age onset RA. This study provides a different genetic susceptibility to the development of RA between younger

![Figure 1](http://ard.bmj.com) Distribution of age at onset of RA in women by Px haplotypes.

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and older onset female RA, suggesting a different pathogenesis is involved in the two subgroups.

It is noteworthy that Px haplotype 11 was found to be a risk factor for osteoporosis, whereas Px haplotype 00 had a preventive role in a previous study. 10 Osteoporosis commonly seen in RA has been thought to be caused by disuse, use of corticosteroids, and rheumatoid inflammation. 16 Our study suggests that the genetic predisposition for RA is also related to osteoporosis in some subgroups of RA.

Loss of ovarian function causes a precipitous loss of bone that can be prevented by oestrogen replacement. Although cellular and biochemical changes mediate the adverse effects of oestrogen deficiency, it has been shown that oestrogen loss up-regulates osteoclastogenesis through an increase in the production of interleukin 6 (IL6) in the microenvironment of the bone tissue, resulting in osteoporosis. 17 On the other hand, the concentration of IL6 is increased in the synovial fluids of RA patients, and IL6 is believed to play important parts in immune response and activation of inflammation at articular joints. 18 It is already known that articular chondrocytes and synovial cells express ORs 19–22 and that oestrogen induces the up-regulation of IL6 production by chondrocytes, showing a conspicuous difference from bone tissue. 23 It is therefore probable that women who show a specific reaction to the hormonal stimuli may tend to develop either osteoporosis in bone tissue at the postmenopausal period or rheumatoid inflammation in joint tissues at the premenopausal period through different effects of oestrogen toward IL6 production.

Interestingly, the age at onset of RA has been increasing in Japan over a 30 year period.24 Previously, women of childbearing age were more often affected, but now the peak has shifted to the perimenopausal age. This increase in age at onset of RA in Japan is probably reducible to some environmental changes that have occurred during these past years. Given that age at onset of RA is influenced by hormonal status, this study suggests that a change in hormonal environment, such as a decrease in childbearing, may explain this phenomenon.

No significant relation between OR genotypes and disease severity indices was found in this study. It is to be noted that the number of seronegative patients and of those with nodules were too small to draw a negative conclusion.

Specific OR genotypes may directly affect the levels of expression through transcriptional regulation, resulting in disease onset, or it is possible that they may be indirectly related to another responsible gene, such as HLA gene, because both the OR and HLA genes are mapped in chromosome 6. The exact mechanism of the relation between OR gene polymorphisms and the age at onset of RA therefore needs to be further investigated.

In this study, determination of the age at onset was based on the patient’s recollection, probably resulting in inclusion of some recall bias. In addition, if p values are corrected for multiple comparison, they are no longer significant. Therefore, the results of this study should not be considered as proof of an association, but as evidence for a putative association that needs to be confirmed in a separate, larger study.

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