EXTENDED REPORTS

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

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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 oestrogen at the onset of the disease.**

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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.[1–3] The onset of symptoms is often associated with menopausal transition.[4] With these clinical observations, the role of sex hormones such as oestrogens in the pathogenesis of RA has been discussed.[5]

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.[6] 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,[7 8] hypertension,[9] osteoporosis,[10] and generalised osteoarthritis[11] 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,[12] 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.[13] 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

	Male RA (n=70)	*Female RA (n=240)*
Age (y)	59.6 (22–82)	56.6 (25–85)
Age at onset (y)	50.3 (18–82)	45.2 (11–81)*
Seropositivity (%)	92.9	92.5
Rheumatoid nodule positivity (%)	12.9	13.3

* p = 0.007.

Table 2 Frequency of OR genotypes and relative risks for developing RA

	Men				*Women*			
OR genotypes	*RA % (n)*	*Control % (n)*	*Odds ratio (95% CI)*	*p value*	*RA % (n)*	*Control % (n)*	*Odds ratio (95% CI)*	*p value*
PPXX	4.3 (3)	3.7 (11)	1.18 (0.32, 4.33)	0.81	2.9 (7)	2.9 (10)	1.02 (0.38, 2.72)	0.97
PPXx	4.3 (3)	8.3 (25)	0.49 (0.14, 1.68)	0.26	8.8 (21)	10.3 (36)	0.84 (0.48, 1.47)	0.54
PPxx	11.4 (8)	5.7 (17)	2.15 (0.89, 5.20)	0.09	6.3 (15)	4.3 (15)	1.49 (0.71, 3.11)	0.29
PpXx	15.7 (11)	23.0 (69)	0.62 (0.31, 1.25)	0.19	22.1 (53)	20.9 (73)	1.08 (0.72, 1.60)	0.72
Ppxx	37.2 (26)	26.3 (79)	1.65 (0.95, 2.86)	0.07	25.0 (60)	26.3 (92)	0.93 (0.64, 1.36)	0.73
ppxx	27.1 (19)	33.0 (99)	0.76 (0.42, 1.35)	0.34	34.9 (84)	35.3 (124)	0.98 (0.70, 1.38)	0.91

95% CI: 95% confidence intervals.

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

	Male RA				*Female RA*			
OR genotypes	*n*	*Age at onset (y)*	*RF (+) % (n)*	*RN (+) % (n)*	*n*	*Age at onset (y)*	*RF (+) % (n)*	*RN (+) % (n)*
PPXX	3	50.0	66.7 (2)	0.0 (0)	7	53.1	85.7 (6)	0.0 (0)
PPXx	3	45.0	100.0 (3)	33.3 (1)	21	42.7	100.0 (21)	4.8 (1)
PPxx	8	52.8	100.0 (8)	25.0 (2)	15	40.5	80.0 (12)	6.7 (1)
PpXx	11	49.8	100.0 (11)	18.2 (2)	53	42.3	92.5 (49)	7.5 (4)
Ppxx	26	51.7	88.5 (23)	11.5 (3)	60	45.6	91.7 (55)	20.0 (12)
ppxx	19	48.5	94.7 (18)	5.3 (1)	84	47.6	94.0 (79)	16.7 (14)
p value		0.94	0.19	0.33		0.047	0.98	0.36

RF: rheumatoid factor, RN: rheumatoid nodule.

eral blood was collected for the analysis of OR gene polymorphisms. The blood samples of 300 men (aged 17–91, mean 48.9) and 350 women (20–87, mean 43.5) who underwent an annual health care check held by several communities were used as a control.

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.[10] Briefly, 100 ng of genomic DNA, extracted from peripheral blood, was amplified by a polymerase chain reaction (PCR) using 2.5 units of Ex Taq polymerase with the accompanying buffer, 2.5 mM each of dNTP (TAKARA SHUZO, Otsu, Japan) and 0.2 μM each of specific oligonucleotide primers in the same PCR conditions.[10] The product contains a part of intron 1 and exon 2 of the OR gene. The amplified PCR products were digested with a restriction endonuclease, Pvu II or Xba I (TAKARA SHUZO) and electrophoresed on 1.0% agarose gel. The RFLPs 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. 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 χ^2 test was also used when appropriate. The software used for the analysis was SPSS (SPSS Inc, Chicago, USA).

Results

CHARACTERISTICS OF RA PATIENTS

Table 1 summarises by sex the clinical characteristics of the RA patients in the study. Their ages ranged from 22 to 82 (mean 59.6) for men and from 25 to 85 (mean 56.6) for women. The age at onset of RA was from 18 to 82 (mean 50.3) for men and from 11 to 81 (mean 45.2) for women, the difference between both sexes being significant (p=0.007). Serum rheumatoid factor positivity was 92.9% for men and 92.5% for women, while rheumatoid nodule positivity was 12.9% for men and 13.3% for women. Frequencies of OR genotypes in overall RA RFLPs of the OR gene were classified into six genotypes according to the combination of both digestion patterns of Pvu II and Xba I: PPXX, PPXx, PPxx, PpXx, Ppxx and ppxx. We could not find the three genotypes, PpXX, ppXX and ppXx in any of the subjects. The frequencies of OR genotypes in RA patients and controls are shown by sex in table 2. The frequencies in both the male and female controls were essentially the same as those previously reported for Japanese women.[10] There was no significant difference between the male

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

	Male RA (n=59)				*Female RA (n=187)*			
Px haplotypes	*n*	*Age at onset*	*RF (+) % (n)*	*RN (+) % (n)*	*n*	*Age at onset*	*RF (+) % (n)*	*RN (+) % (n)*
11	8	52.8	100.0 (8)	25.0 (2)	15	40.5	80.0 (12)	6.7 (1)
10	29	51.0	89.7 (26)	13.8 (4)	81	44.8	93.8 (76)	16.0 (13)
00	22	48.7	90.9 (20)	4.5 (1)	91	48.0	93.4 (85)	15.4 (14)
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.

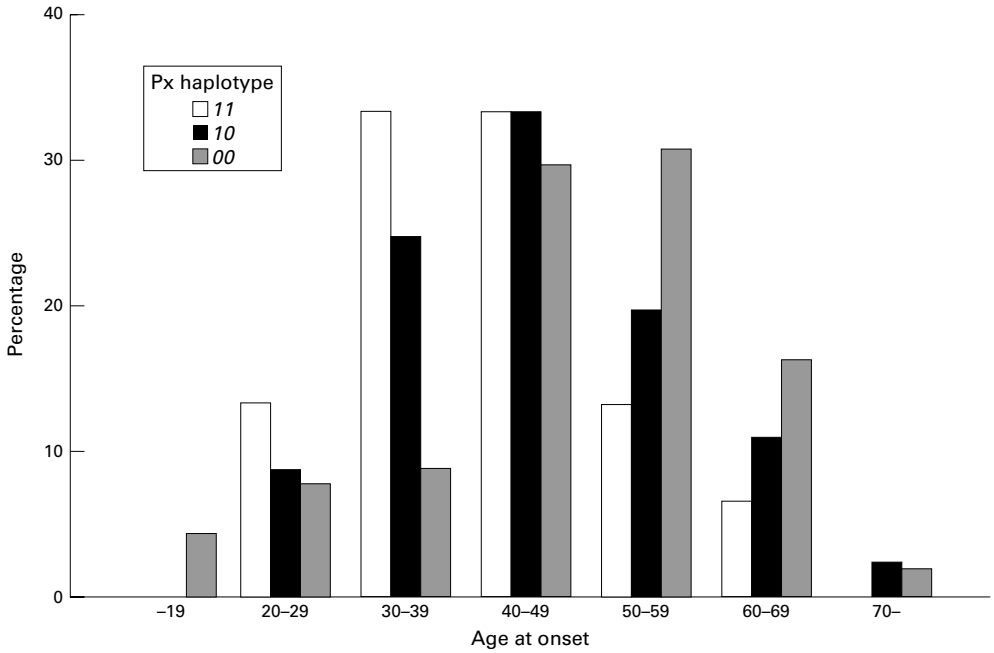

Figure 1 Distribution of age at onset of RA in women by Px haplotypes.

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 χ^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. Figure 1 shows the distribution of age at onset for these three groups. The peak age at onset in patients having haplotype *11* is seen at the fourth and fifth decade, in those having *10* at the fifth decade, and in those having *00* at the fifth and sixth decade.

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

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 OR[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 most 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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1 Lawrence JS. *Rheumatism in populations*. London: Heinemann, 1977.
2 Persellin RH. The effect of pregnancy on rheumatoid arthritis. Bull Rheum Dis 1976;27:922–7.
3 Rudge SR, Kowanko IC, Drury PL. Menstrual cyclicity of finger joint size and grip strength in patients with rheumatoid arthritis. Ann Rheum Dis 1983;42:425–30.
4 Goemaere S, Ackerman C, Goethals K, De Keyser F, Van der Straeten C, Verbruggen G, *et al*. Onset of symptoms of rheumatoid arthritis in relation to age, sex and menopausal transition. J Rheumatol 1990;17:1620–2.
5 Masi AT. Sex hormones and rheumatoid arthritis: cause or effect relationships in a complex pathophysiology? Clin Exp Rheumatol 1995;13:227–40.
6 Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, *et al*. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol 1993;32:903–7.
7 Hill SM, Fuqua SAW, Chamness GC, Greene GL, McGuire WL. Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism. Cancer Res 1989;49: 145–8.
8 Yaich L, Dupont WD, Cavener DR, Parl FF. Analysis of the Pvu II restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. Cancer Res 1992;52:77–83.
9 Lehrer S, Rabin J, Kalir T, Schachter BS. Estrogen receptor variant and hypertension in women. Hypertension 1993; 21:439–41.
10 Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 1996;11: 306–11.
11 Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I, Hukuda S. Estrogen receptor gene polymorphism and generalized osteoarthritis. J Rheumatol 1998;25:134–7.
12 Cutolo M, Sulli A, Seriolo B, Accardo S, Masi AT. Estrogens, the immune response and autoimmunity. Clin Exp Rheumatol 1995;13:217–26.
13 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
14 Inoue K, Shichikawa K, Nishioka J, Hirota S. Older age onset rheumatoid arthritis with or without osteoathritis. Ann Rheum Dis 1987;46:908–11.
15 Terkeltaub R, Esdaile J, Décary F, Tannenbaum H. A clinical study of older age onset rheumatoid arthritis with comparison to an early onset group. J Rheumatol 1983;10:418–24.
16 Deodhar AA, Woolf AD. Bone mass measurement and bone metabolism in rheumatoid arthritis: a review. Br J Rheumatol 1996;35:309–22.
17 Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, *et al*. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 1992; 257:88–91.
18 Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y. Effects of interleukin-6 on the metabolism of connective tissue components in rheumatoid synovial fibroblasts. Arthritis Rheum 1992;35:1197–201.
19 Young PCM, Stack MT. Estrogen and glucocorticoid receptors in adult canine articular cartilage. Arthritis Rheum 1982;25:568–73.
20 Rosner IA, Manni A, Malemud CJ, Boja B, Moskowitz RW. Estradiol receptors in articular chondrocytes. Biochem Biophys Res Commun 1982;106:1378–82.
21 Cutolo M, Accardo S, Villaggio B, Clerico P, Bagnasco M, Coviello DA, *et al*. Presence of estrogen-binding sites on macrophage-like synoviocytes and CD8+, CD29+, CD45RO+ T lymphocytes in normal and rheumatoid synovium. Arthritis Rheum 1993;36:1087–97.
22 Ushiyama T, Inoue K, Nishioka J. Expression of estrogen receptor related protein (p29) and estradiol binding in human arthritic synovium. J Rheumatol 1995;22:421–6.
23 Guerne PA, Carson DA, Lotz M. IL-6 production by human articular chondrocytes. J Immunol 1990;144:499–505.
24 Imanaka T, Shichikawa K, Inoue K, Shimaoka Y, Takenaka Y, Wakitani S. Increase in age at onset of rheumatoid arthritis in Japan over a 30 year period. Ann Rheum Dis 1997;56:313–16.