Association between Estrogen Receptor Polymorphism with Pain Susceptibility in Female TMJ Osteoarthritis Patients

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2010년 7월

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Abstract

Association between Estrogen Receptor Polymorphism with Pain Susceptibility in Female TMJ Osteoarthritis Patients

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The purpose of this study was to investigate the possible association between estrogen receptor alpha (ERα) polymorphism and pain susceptibility in female symptomatic temporomandibular joint (TMJ) osteoarthritis (OA) patients.

One hundred women, diagnosed as TMJ OA according to the research diagnostic criteria for temporomandibular disorders (RDC-TMD), were selected as a patient group, and 74 women with no sign and symptom of temporomandibular disorder (TMD) were assigned to a control group. *Pvu II* and *Xba I* restriction fragment length polymorphisms (RFLP) were analyzed by direct haplotyping. To determine the presence of an association between ERα polymorphism and TMJ pain intensity, the patient group was divided into three

subgroups according to visual analogue scale(VAS): Mild pain (0≤VAS<4); Moderate pain (4≤VAS<7); Severe pain (7≤VAS≤10). Frequencies of genotypes and haplotypes in the patient and control groups were compared, and the association between pain intensity and copy numbers of PX haplotype were evaluated using the chi-square test.

No significant differences in genotype and haplotype frequencies were observed between the patient and control groups ($p>.05$). However, patients (TMJ OA patients) carrying PX haplotype were found to have a significantly higher risk of moderate or severe pain compared to the patients without PX haplotype, suggesting that ERα polymorphism may be associated with pain susceptibility in female TMJ OA patients.

Keywords : Estrogen receptor alpha (ERα), polymorphism, temporomandibular joint (TMJ), osteoarthritis (OA), pain

Association between Estrogen Receptor Polymorphism with Pain Susceptibility in Female TMJ Osteoarthritis Patients

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Ⅰ. Introduction

Osteoarthritis (OA) is known as the most common form of arthritis presented with characteristics of focal, progressive hyaline articular cartilage

loss along with development of osteophytes and/or bony sclerosis[10]. Temporomandibular joint (TMJ) OA is the most common form of arthritis developing in TMJ, and presents pain, crepitus or multiple joint noises during function, radiographic structural bony change, and joint space narrowing, which may not be evident during the early disease stage[1]. OA can be classified as primary (unknown causes) and secondary (local and systemic causes). Primary OA is considered idiopathic due to the absence of identifiable local or systemic etiologic factor. In secondary OA, systemic causes are related to ethnicity, hormonal status, nutritional factors, genetics, and bone metabolism[10], whereas local causes include obesity, mechanical environment, over-loading of articular cartilage, and acute joint injury[10].

Hormones have been proposed to define host adaptive capacity of TMJ[3], and estrogen has been reported to be associated with osteoarthritis by a number of investigatiors[5,19,21,22]. Estrogen performs its biologic function through estrogen receptors, i.e., estrogen receptor alpha (ERα) and beta (ERβ), which have different localizations and concentrations within the body. In particular, ERα has been identified in articular cartilage, growth plate, and TMJs[2,4,6].

A number of studies have been conducted on the relationship between sex hormones and temporomandibular disorder (TMD). Yasuoka et al. reported that while excessive exogenous hormones are found in women with TMD, endogenous hormones may play a significant role in TMJ remodeling[24], whereas Marcus suggested that drop in estrogen may be associated with changes in neurotransmitters responsible for migraine. Based on these findings, it has been speculated that neurotransmitter change associated with estrogen change might influence other pain conditions. Furthermore, LeResche

et al. reported that TMD pain was highest when estrogen level in menstrual cycle was lowest, and rapid estrogen change may also be associated with increased pain[13]. However, the role of estrogen receptor polymorphisms in evoking pain has not been elucidated except for ERβ, in which the cytosine-adenine repeat polymorphism of the ERβ gene has been reported to be associated with menopausal and premenstrual symptoms[20].

The purpose of this study was to investigate the possible association between ERα polymorphism and OA pain susceptibility in symptomatic female TMJ OA patients.

Ⅱ. Materials and Methods

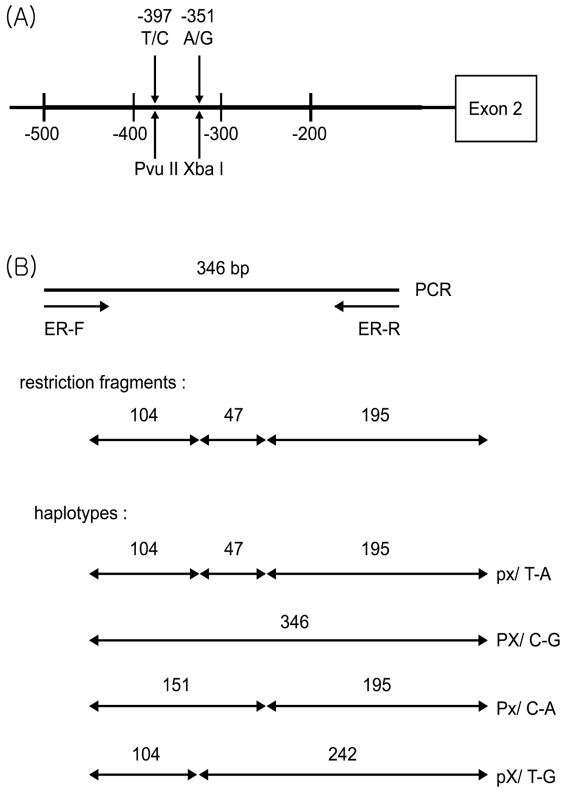
Consecutively, 100 women (aged 17-48, mean age 28.9±9.74) who visited TMJ and Orofacial Pain Clinic, Department of Oral Medicine, College of Dentistry, Yonsei University, and who were diagnosed as symptomatic TMJ OA according to research diagnostic criteria for temporomandibular disorders (RDC-TMD)[8] were assigned as the patient group. Those with trauma history, systemic endocrine disturbances, history of psychoactive drug medication, rheumatoid arthritis were excluded. Seventy-four age matched women (aged 21-42, mean age 25.57±5.48), composed of dental students and staff members at the College of Dentistry, Yonsei University, with no history of TMD and who volunteered for this study, were selected as controls._The purposes of the study and the study protocol were fully explained, and signed written consent was obtained from all subjects who participated in this study, as required by the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board at Yonsei Universtiy.

1. Genotyping

Genomic DNA was extracted from buccal mucosa using QiaAmp Mini Kit(Qiagen GmbH, Hilden, Germany). 346 base pair polymerase chain reaction (PCR) fragment of ERα was generated using a primer pair (forward, 5'-GATATCCAGGGTTATGTGGCA-3', and reverse, 5'-AGGTGTTGCCTATTATATTAACCTTGA-3') in a 20㎕ reaction mixture containing 20ng genomic DNA. PCR products were collected after 30 amplification cycles {94℃, 60℃, 72℃ for 45 seconds each}. A direct haplotyping

method was used to analyze *Pvu Ⅱ* and *Xba I* RFLP fragments as described in a previous study[12]. 5㎕ of PCR products were digested using 2 units of *Pvu Ⅱ* and 2 units of *Xba I* restriction enzymes, and incubated for 90 minutes at 37℃. Digestion products were analyzed by electrophoresis in a 2% agarose gel for 45 minutes at 50 V. All genotypes were scored separately by two individuals, and equivocal genotypes were retyped. The absence or presence of *Pvu Ⅱ* and *Xba I* restriction sites are indicated by P or p and X or x, respectively (fig. 1).

Fig. 1.



2. Assessment of pain

Pain intensity was measured using Visual Analogue Scale(VAS), where a score of 0 indicates pain free state and a score of 10 indicates worst imaginable pain. VAS scores used in the analysis were recorded by patients when they first visited our clinic. To analyze the association between PX haplotype and pain intensity, the patient group was divided into three clinical subgroups according to VAS; Mild pain ($0 \leq VAS < 4$); Moderate pain ($4 \leq VAS < 7$); Severe pain ($7 \leq VAS \leq 10$). Individuals carrying one or more PX haplotype (PPXX, PPXx, PpXx) were assigned to genotype 1, and those lacking PX haplotype (PPxx, Ppxx, ppxx) were assigned to genotype 0.

3. Statistical analysis

Comparisons of frequencies of genotypes and haplotypes between the patient and control groups were performed, and the association between the pain intensity and PX haplotype copy number was evaluated using the chi-square test. Window SAS v.8.1 (SAS institute. Inc., USA) was used for all analyses. P-values of less than 0.05 were considered statistically significant. 14 patients without VAS evaluation records were excluded from the analysis of the relationship between pain intensity and PX haplotype copy number.

Ⅲ. Results

Using the direct haplotyping method, three different haplotypes and six different genotypes, determined by combinations of haplotypes, were identified, and their frequencies in the patient and control groups are presented in Table 1. No significant differences were observed between the patient and control groups in terms of haplotype and genotype frequencies ($p > .05$, Table 1). The fourth possible haplotye, pX, was not observed as has been reported previously[5,12,21].

Table 1. Frequencies of ERα genotypes and haplotypes in the study versus control groups.

ERα		Study group (n=100) n (%)	Control group (n=74) n (%)
Genotypes*	PPXX	4 (4)	0 (0)
	PPXx	6 (6)	10 (13.5)
	PPxx	4 (4)	3 (4.1)
	PpXx	25 (25)	18 (24.3)
	Ppxx	21 (21)	14 (18.9)
	ppxx	40 (40)	29 (39.2)
Haplotypes**	PX	39 (19.5)	28 (18.9)
	Px	35 (17.5)	30 (20.3)
	px	126 (63.0)	90 (60.8)

*: p = 0.3707 , **: p = 0.8065

Associations between ERα genotypes and pain intensity in the patient group are presented in Table 2. The presence of PX haplotype in patients was significantly associated with perceived pain intensity (p=.0382). Patients with the PX haplotype (genotype 1) were found to have a significantly higher risk of moderate or severe pain with odds ratios (OR) of 3.91(1.15-13.34, p=.0295, 95% confidence interval (CI)) and 5.11(1.23-21.28, p=.025, 95% CI), respectively (Table 2), compared to patients without the PX haplotype.

Table 2. Association between the copy numbers of PX haplotype and pain intensity in the study group.

Pain Intensity	Genotype 1[a] cases/total (%)	Genotype 0[b] cases/total (%)	OR (95% C.I)
Mild pain (0≤VAS<4)	4/29 (13.8)	23/57 (40.3)	1
Moderate pain (4≤VAS < 7)	17/29 (58.6)	25/57 (43.9)	3.91 (1.15-13.34)*
Severe pain (7≤VAS≤10)	8/29 (27.6)	9/57 (15.8)	5.11 (1.23-21.28)**

OR = Odds Ratio C.I = Confidence Interval

Chi-square distribution is significant (6.53, degrees of freedom=2, p-value=0.0382) which confirms the association between the presence of PX haplotype and pain intensity.

*; p = 0.0295, **; p = 0.0250

[a] Subjects carrying 1 or 2 copies of PX haplotype

[b] Subjects carrying no copy of PX haplotype

14 subjects whose records of VAS evaluation were missing or non-existent from the beginning were not included. 86 subjects (29 with Genotype 1 and 57 with Genotype 0) were included for analysis.

Ⅳ. Discussion

The relationship between ERα and osteoarthritis has been investigated on a number of occasions. However, associations between the two remain controversial. Ushiyama et al. found that ERα genotype PpXx is significantly related to generalized OA[22], and also reported that rheumatoid arthritic women with PPxx genotype (homozygote of Px) tended to have developed rheumatoid arthritis at a younger age than those lacking Px haplotype[21]. Bergink et al. found that haplotype PX was associated with an increased prevalence of radiographic knee OA[5], but Loughlin et al. found no relation between ERα gene polymorphisms and OA in a Caucasian population[15]. Jin et al. also found no association between ERα gene polymorphisms and primary knee OA in a Korean cohort[9]. However, to the best of our knowledge, no report has been issued concerning ERα polymorphisms in patients with TMJ OA, except for our previous study, in which no significant differences between ERα genotype and haplotype distributions were found between TMJ OA patients and controls[12], which is confirmed by the present study.

Pain, defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage[17], is felt and expressed to different degrees. Evidence for differences in pain perception by sex has been presented, and gonadal hormones, for example, have been proposed to play a significant role in the modulation of pain and analgesia in animals and humans[7]. In addition, genetic epidemiologic approaches to the search for genes associated with various types of pain have been conducted in an

attempt to understand the nature of pain and as a result, genetic polymorphisms of interleukin-6 has been implicated in the different sensitivities to pain[18].

The present study demonstrates an association between the PX ERα haplotype and TMJ pain intensity. Of the three ERα haplotypes present (PX, Px, and px), patients carrying one or more PX haplotypes showed 3.9- and 5.1 -fold higher risks of experiencing moderate or severe pain, respectively, compared with patients without the PX haplotype with statistical significance. This result suggests that ERα gene polymorphisms may be associated with pain susceptibility in TMJ OA patients, which supports the results of previous studies which suggested that hormones affect TMD pain[11,14,23]. The clinical implication of this result is that patients heterozygous or homozygous for the PX haplotype may require more intensive treatment for TMD, e.g., physical therapy, pharmacotherapy, occlusal stabilization splint therapy, and others.

In conclusion, we speculate that the ERα polymorphisms, especially the PX haplotype, may be involved in pain perception in symptomatic TMJ OA. In our previous study[12], the Px haplotype was found to be associated with an altered mandibular dimension. Therefore, ERα polymorphisms appear to contribute to mandibular dimension and pain susceptibility in female symptomatic TMJ OA patients, but different haplotypes are involved.

However, the present study is limited by a small sample size, and inherent subjective bias associated with the use of VAS to assess pain severity. Another point to be clarified in the present study is that the degree of OA change was not considered because TMJ OA was diagnosed clinically by the presence of joint crepitus and pain as described by the RDC-TMD[8]. Further

studies regarding the association between ER polymorphisms and degree of OA change are warranted. Furthermore, another approach involving the enrollment of a control group composed of TMJ patients without OA change but with TMJ pain is needed to evaluate the interrelationship among ER polymorphisms, OA change and pain. Further genetic epidemiologic researches to clarify the underlying mechanism of estrogen and its receptors related to pain processes are also required to assess the need for pain treatment in TMJ OA patients, and may explain why women suffer more from TMJ pain than men.

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국문요약

측두하악관절 골관절염 여성 환자의 통증 감수성과 에스트로겐 수용체 유전자 다형성과의 연관성

본 연구의 목적은 에스트로겐 알파 수용체 유전자 다형성과 측두하악관절의 골관절염이 있는 여성 환자의 통증에 대한 민감성과의 연관성에 대해 알아보는 것이었다.

측두하악관절장애 진단기준에 따라 측두하악관절의 골관절염으로 진단된 100명의 여성을 실험군으로, 그리고 측두하악관절장애의 증상이나 징후가 없는 74명의 여성을 대조군으로 선택하였다. 직접 하플로타잎 (direct haplotyping) 방법을 이용하여 *Pvu II* 와 *Xba I* 제한단편장다형화현상을 분석하였다. 실험군을 시각적 상사 척도 (visual analog scale; VAS)에 따라 3 군으로 분류하였다: 경도의 통증 ($0 \leq VAS < 4$); 중등도의 통증 ($4 \leq VAS < 7$); 중증도의 통증 ($7 \leq VAS \leq 10$). 실험군과 대조군간의 유전자형과 하플로타잎의 빈도를 비교하였고, 카이제곱 검사를 이용하여 통증의 민감성과 PX 하플로타잎의 개수간의 연관성을 평가하였다.

실험군과 대조군간에 유전자형과 하플로타잎의 빈도는 유의한 차이가 발견되지 않았다 ($p > .05$). 실험군에서 PX 하플로타잎을 가지고 있는 환자가 PX 하플로타잎을

가지고 있지 않은 환자에 비해 중등도 또는 중증도의 통증을 나타낼 위험도가 유의하게 높은 것으로 나타났다. 즉, 이 결과는 에스트로겐 알파 수용체의 유전자 다형성이 측두하악관절의 골관절염이 있는 여성 환자의 통증 민감성과 연관성이 있을 수 있음을 시사한다.

핵심되는 말 : 에스트로겐 알파 수용체, 유전자 다형성, 측두하악관절, 골관절염, 통증