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Short Communication

Estrogen receptor-α gene polymorphisms in patients with periodontitis

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Background: Recent studies have shown that estrogen receptor-α (ER-α) gene polymorphisms are associated with alterations in bone mineral density and osteoporosis. It is unclear whether ER-α gene polymorphisms are associated with alveolar bone loss in patients with periodontitis.

Objective: The aim of this study was to investigate the relationship between ER-α gene polymorphisms and periodontitis.

Methods: Ninety patients with aggressive periodontitis, 34 patients with chronic periodontitis and 91 healthy controls were recruited in this study. All these subjects belonged to the Han Chinese race. DNA was extracted from peripheral blood of each subject. The ER-α gene was analyzed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) with *Pvu*II and *Xba*I restriction endonucleases.

Results: The detection frequency of XX genotype was significantly higher in the chronic periodontitis patients than in the healthy controls (11.8% vs. 4.4%, $p < 0.05$). The difference between the female chronic periodontitis patients and healthy controls (26.7% vs. 2.1%, $p < 0.01$) was statistically significant, but no difference was found between the male patients and controls ($p > 0.05$). The detection frequency of *Pvu*II ER genotypes was not statistically different among the three groups ($p > 0.05$).

Conclusions: In female Han Chinese population, the XX genotype may be a risk indicator for chronic periodontitis.

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Periodontitis is an inflammatory disease and also one of the most familiar bone diseases (1), characterized by loss of connective tissue and alveolar bone (2). In the later stages of this disease, tooth mobility and abscess may occur, eventually leading to tooth loss. Although the causative agent in periodontitis is pathogenic bacterial plaque, the disease severity partially depends on the susceptibility of the host. Osteoporosis is characterized by reduction in bone mass, resulting in skeletal fragility and fracture. Kribbs reported that mandibular bone mass was less and cortex at the gonion was thinner in osteoporotic women than in non-osteoporotic women (3). Recently, 70 postmenopausal women with periodontitis were studied by Wactawski-Wende *et al.* (4) and positive correlations were seen between alveolar bone loss and bone mineral density at the spine, trochanter, Ward's triangle and total femur. It was postulated that some host factors associating with bone metabolism might contribute to susceptibility to periodontitis. Studies relating vitamin D receptor gene polymorphism and periodontitis (5, 6) provided more strong evidence for this association.

Estrogen is a hormone that regulates bone metabolism. Its special receptor is estrogen receptor (ER). There are two isoforms of the human ER, named ER-α and ER-β, each with distinct tissue and cell patterns of expression. ER-α has been found being expressed in osteoblasts, osteoblast-like cells (7), osteoclasts (8) and bone cells (9). It has been proven that *Pvu*II and *Xba*I

restriction fragment length polymorphisms (RFLP) of the ER-α gene were associated with altered bone mineral density and osteoporosis (10–12). To our knowledge, there are no reports about the relationship between polymorphisms of the ER-α gene and periodontitis.

In order to search for the relationship between ER-α gene polymorphism and periodontitis in Chinese, the ER-α gene polymorphisms of aggressive periodontitis, chronic periodontitis and healthy controls were detected in our study.

Materials and methods

Selection of subjects

Ninety aggressive periodontitis (37 males, 53 females) and 34 chronic periodontitis (19 male, 15 female) patients were recruited from the Periodontology Clinic in Peking University School of Stomatology. Aggressive periodontitis was defined according to the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999 (13). Patients in the aggressive periodontitis group were 14–39 years old (mean age 29.0 ± 6.3); patients in the chronic periodontitis group were 18–58 years old (mean age 38.0 ± 10.4). In the present study, there were only four patients with localized aggressive periodontitis; the other 86 patients had generalized aggressive periodontitis. Because of the small number of localized aggressive periodontitis patients, the aggressive periodontitis group was not divided. All periodontitis patients presented with probing depths of at least 5 mm at two sites in each quadrant with radiographic evidence of alveolar bone loss. But, the percentages of the teeth with bone absorption more than 1/3 of the root length were 64% in the aggressive periodontitis group and 22% in the chronic periodontitis group. Ninety-one healthy control subjects, aged 18–55 years old (mean age 29.1 ± 6.2, 43 males, 48 females) with no previous or existing evidence of periodontal disease were selected from the staff and students of the Peking University School of Stomatology. All subjects belonged to the Han race, which comprises the majority of the Chinese population. All study subjects were free from any systemic disease and were not under any medication known to affect the periodontal status. Pregnant females were also excluded. All of them signed an informed consent to participate in the study.

Analysis of estrogen receptor-α genotypes

Peripheral blood samples were collected from the subjects by venipuncture and anti-coagulated with EDTA. Genomic DNA was extracted from white blood cells of each subject using the DNA Blood Mini Kit (Huashun Biotechnology Co. Ltd, Shanghai, People's Republic of China), following the manufacturer's instructions.

DNA fragments including *Pvu*II and *Xba*I restriction sites in intron 1 were amplified by polymerase chain reaction (PCR). Genomic DNA was amplified in 50 μl of a buffer solution: 250 μmol/l of each four deoxyribonucleotides, 10 mmol/l Tris-HCl, 50 mmol/l KCl, 10 mmol/l $(NH_4)_2SO_4$, 2 mmol/l $MgCl_2$, 2 U *Taq* DNA polymerase and 0.2 μmol/l of each oligonucleotide primers (forward: 5′-CTGCCACCCT-ATCTGTATCTTTTCCTATTCTCC-3′ and reverse: 5′-TCTTTCTCTGCCA-CCCTGGCGTCGATTATCTGA-3′). The thermocycling was performed as following: 95°C for 5 min followed by 95°C for 30 s, 60°C for 30 s, 72°C for 90 s, for 35 cycles, and 72°C for 7 min. The final PCR product was 1.3 kb. The PCR product was digested with restriction endonuclease (*Pvu*II and *Xba*I; TaKaRa Biotechnology Dalian Co. Ltd, Dalian, China), electrophoresed in 1% agarose gel, and determined by ethidium bromide/UVB illumination. Presence and absence of the restriction endonuclease site were represented as small letters (p, x) and capital letters (P, X), respectively. Digestion revealed fragments of 0.85 kb and 0.45 kb for the pp genotype and 0.9 kb and 0.4 kb for xx genotype.

Statistical methods

The distributions of genotypes frequencies in the diseases and control groups were compared using standard chi-squared tests. A *p*-value of less than 0.05 indicated statistical significance.

Results

Distribution of the ER-α genotypes

In the total detected samples, the frequency of XX, Xx and xx genotypes was 6.5%, 54.4% and 39.1%, and the frequency of PP, Pp and pp was 23.3%, 46.5% and 30.2%. The distribution of the complex ER-α genotypes of *Xba*I and *Pvu*II is shown in Table 1. PpXx was the major genotype in the total samples, but no samples with ppXX genotype were detected.

*Pvu*II ER-α genotype polymorphism in periodontitis

The detection frequency of PP, Pp and pp genotypes was 25.3%, 44.0% and 30.7% in the controls, 22.2%, 52.2% and 25.6% in the aggressive perio-

Table 1. The distribution of the complex estrogen receptor-α genotypes of XbaI and PvuII

	Genotypes								
	PPXX	PPXx	PPxx	PpXX	PpXx	Ppxx	ppXx	ppxx	Total
Control	3	12	8	1	28	11	14	14	91
	3.3%	13.2%	8.8%	1.1%	30.8%	12.1%	15.4%	15.4%	
AgP	5	10	5	1	32	14	10	13	90
	5.6%	11.1%	5.6%	1.1%	35.6%	15.6%	11.1%	14.4%	
CP	2	3	2	2	7	4	1	13	34
	5.9%	8.8%	5.9%	5.9%	20.6%	11.8%	2.9%	38.2%	
Total	10	25	15	4	67	29	25	40	215
	4.7%	11.6%	7.0%	1.9%	31.2%	13.5%	11.6%	18.5%	

AgP, aggressive periodontitis; CP, chronic periodontitis.

dontitis patients, and 20.6%, 38.2% and 41.2% in the chronic periodontitis patients. There was no statistical difference in the distributions of *Pvu*II ER-α genotypes between groups ($p > 0.05$).

*Xba*I ER-α genotype polymorphism in periodontitis

The frequency of XX, Xx and xx genotypes was 4.4%, 59.3% and 36.3% in the controls and 6.7%, 57.8% and 35.5% in the aggressive periodontitis patients. There was no statistical difference between these two groups ($p > 0.05$). The frequency of XX, Xx, xx genotypes in the chronic periodontitis patients (11.8%, 32.4% and 55.8%) was significantly different from the controls (4.4%, 59.3% and 36.3%, $p < 0.05$) (Table 2), especially when considering the female chronic periodontitis patients and healthy controls (26.7%, 26.7% and 46.6% vs. 2.1%, 64.6% and 33.3%, $p < 0.01$) (Table 3). However, no difference was found between the male chronic periodontitis patients and controls ($p > 0.05$) (Table 4). There was no significant difference between the female aggressive periodontitis patients and controls either ($p > 0.05$) (Table 5).

Table 2. Distribution of XbaI estrogen receptor-α genotypes in the chronic periodontitis patients and controls

	Genotypes (%)			
	XX	Xx	xx	Total
Control	4 (4.4)	54 (59.3)	33 (36.3)	91
Chronic periodontitis	4 (11.8)	11 (32.4)	19 (55.8)	34

$\chi^2 = 7.842$, $p = 0.020$.

Table 3. Distribution of XbaI estrogen receptor-α genotypes in female chronic periodontitis patients and controls

	Genotypes (%)			
	XX	Xx	xx	Total
Control (female)	1 (2.1)	31 (64.6)	16 (33.3)	48
Chronic periodontitis (female)	4 (26.7)	4 (26.7)	7 (46.6)	15

$\chi^2 = 11.010$, $p = 0.004$.

Table 4. Distribution of XbaI estrogen receptor-α genotypes in male chronic periodontitis patients and controls

	Genotypes (%)			
	XX	Xx	xx	Total
Control (male)	3 (7.0)	23 (53.5)	17 (39.5)	43
Chronic periodontitis (male)	0 (0)	7 (36.8)	12 (63.2)	19

$\chi^2 = 4.480$, $p = 0.106$.

Table 5. Distribution of XbaI estrogen receptor-α genotypes in female aggressive periodontitis patients and controls

	Genotypes (%)			
	XX	Xx	xx	Total
Control (female)	1 (2.1)	31 (64.6)	16 (33.3)	48
Aggressive periodontitis (female)	5 (9.4)	28 (52.8),	20 (37.8)	53

$\chi^2 = 3.261$, $p = 0.196$.

Discussion

The gene map locus of ER-α gene is 6q25.1. It is more than 140 kb long, contains eight exons and seven introns, and has five functional domains, designated A/B–F (14). The two RFLPs examined in the present study are located in the A/B domain. The *Pvu*II RFLP site is sequenced in the first intron, 0.4 kb upstream from exon 2, with a single nucleotide polymorphism (T → C) (15) and the *Xba*I RFLP site is approximately 50 bp downstream from the known *Pvu*II site, with a single nucleotide polymorphism (A → G) (16). Although a number of studies have demonstrated the association between genetic polymorphism and bone mass, the findings were inconsistent. Some studies reported an association of ER-α gene polymorphism with bone mass (10–12, 17), whereas others failed to find an association (18–21). The PPxx genotype was responsible for low lumbar spine bone mineral density in Japanese (10) and Chinese (17); however, the PP or xx genotype was associated with higher bone mineral density in American Caucasians (12). The differences among study populations in terms of race, age and environment may explain the inconsistency in results. The distributions of the genotypes in this study were similar to the previous studies, especially the low proportion of the XX genotype detection (Table 6). In a study including 99 healthy postmenopausal Han Chinese women, the lowest bone mineral density was found in subjects with XX genotype and the highest bone mineral density was found with xx genotype (22). The present study demonstrated that the frequency of XX genotype was significantly higher in the chronic periodontitis patients than in the healthy controls.

Estrogen is one of the sex hormones. Females are more likely to experience hormonal imbalance throughout their lives than males. Several studies showed that the polymorphism in the

Table 6. The distributions of different genotypes of estrogen receptor-α reported by different authors

Population	Total number	Genotypes (%)						Reference
		PP	Pp	pp	XX	Xx	xx	
Chinese	215	23.3	46.5	30.2	6.5	54.4	39.1	[a]
Chinese	99	13.1	61.6	25.3	7.1	48.5	44.4	22
Chinese	450	14.0	49.8	36.2	6.4	35.0	58.6	33
Japanese	238	19.3	51.3	29.4	2.9	32.4	64.7	10
Japanese	173	–	–	–	3.5	28.3	68.2	11
Caucasian	90	22.2	44.5	33.3	8.9	40.0	51.1	34

[a]The present study.

ER-α was related to gender-associated disease, breast cancer (15, 23, 24), and was also a risk factor for miscarriage (25). In addition, when the influence of gender on periodontal disease was studied, females were considered more prone than males (26). It was postulated that the effects of the ER-α polymorphisms on periodontitis might be different between males and females. So we divided each patient group according to the gender. It was remarkable that the female chronic periodontitis and healthy subjects exhibited a more significantly statistical difference, whereas no difference was found between male patients and controls. The difference of the frequency of XX genotype between the chronic periodontitis patients and the healthy controls was mainly attributed to the difference in females.

The currently accepted classification of periodontal disease acknowledges the influence of endogenously produced female sex steroid hormones on the periodontium (13). It has been evidenced that the level of estrogen can influence gingival inflammation (27). However, it is still unclear how periodontitis is affected by the intronic polymorphisms of the ER-α gene. Although numerous studies have addressed the relation between polymorphisms of ER-α gene and osteoporosis, little is known about the effect on bone metabolism. A mutation in the exon of the ER-α gene was shown to result in severe osteoporosis in both female and male mice (28). It was postulated that the biological significance of the intronic polymorphisms in the ER-α gene may lie in the regulation of ER-α mRNA levels directly or indirectly. More comprehensive studies in this field may help us find the solution to the above problems and enable us to elucidate the role of host susceptibility in periodontitis.

Recent investigations of periodontal disease focused on identifying possible candidate genes that may modify host immune responses. The IL-1 gene was one of the most studied genes. Some polymorphisms of the IL-1 gene cluster were associated with more severe forms of chronic periodontitis (29, 30). In addition, the polymorphisms of Fc γ receptor gene were associated with additional risk of bone loss in chronic periodontitis (31). A hyperresponsive monocyte phenotype that produces more prostaglandin E_2 (PGE_2) in response to lipopolysaccharide compared to common phenotypes was implicated as a possible factor that increases host susceptibility in localized aggressive periodontitis patients (32). The finding of the present research relating the ER-α gene polymorphisms with chronic periodontitis in females provided a new point of view to choose candidate genes for periodontitis.

In conclusion, the XX genotype may correlate to the susceptibility of chronic periodontitis in female Han Chinese, but not to the susceptibility of aggressive periodontitis. No association of *Pvu*II RFLP of ER-α with periodontitis was found.

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