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Original Article

Polymorphisms in the gene encoding estrogen receptor alpha are associated with osteoarthritis in Han Chinese women

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Abstract: Polymorphisms in the Xba I and Pvu II restriction enzyme recognition sites in the estrogen receptor-alpha gene (*ESR1*) have been associated with multiple diseases, including osteoarthritis. To determine whether such polymorphisms are associated with osteoarthritis in a Han Chinese population, 98 women with osteoarthritis and 196 healthy women were genotyped by PCR-RFLP of *ESR1* with Xba I and Pvu II. Absence of a restriction polymorphism is indicated as an X or P allele; presence of the restriction polymorphism is indicated as an x or p allele. Clinical information was collected on each participant, including body weight, body mass index (BMI), knee radiograms, and bone mineral density (BMD). Body weight and BMI were higher for each Xba I genotype (all $P < 0.05$) in individuals with osteoarthritis compared to controls ($p < 0.05$). Femoral BMD was also significantly higher in the osteoarthritis group ($p < 0.05$). Additionally, the xx genotype for *ESR1* was a significant risk factor for osteoarthritis (*OR*=1.98, 95% *CI*: 1.13~4.20, p=0.036). Thus, consistent with findings in other populations, the estrogen receptor genotype xx appears to be associated with susceptibility to osteoarthritis among Han Chinese women.

Keywords: Osteoarthritis, estrogen, estrogen receptor, Xba I, Pvu II

Introduction

Osteoarthritis (OA), also known as osteoarthropathy, hypertrophic arthritis, degenerative arthritis, or senile arthritis, is a degenerative disorder of the joints that occurs commonly in middle-aged and older people, especially women [1]. Affected individuals gradually develop joint pain, tenderness, rigidity, joint swelling, limitation of movement, and joint deformity. OA has a high prevalence, and in some countries has been identified by radio-imaging in 63% of women over age 65 [2]. In China, radio-imaging has indicated a prevalence of 29.5% in women over age 60, but clinical diagnosis extends that prevalence to~39% [3]. OA not only carries a high morbidity, but can also cause disability, thereby increasing its impact on patients, caregivers, and medical costs [4]. Despite these concerns, the pathogenesis of OA remains unclear.

Current hypotheses propose that OA results from a combination of factors, rather than simply reflecting the aging process. Indeed, OA is correlated with age, mechanical features, obesity, endocrine disorders, and genetic susceptibility [5]. OA has been linked with a number of genes and single nucleotide polymorphisms [5, 6]. Since OA is more prevalent in women than men, it is perhaps unsurprising that variants of the estrogen receptor gene (*ESR1*) are associated with OA [7-9]. Binding of estrogens to ERs can protect the articular cartilage [8], thereby retarding and even preventing the development of OA. Alterations in that binding ability, then, may promote OA.

A previous study assessed the association of two restriction fragment length polymorphisms (RFLPs) in *ESR1* with the prevalence of OA in a population of Japanese women. The study found that women carrying both the Pvu II and Xba I RFLPs were at higher risk of OA [9]. Here, the RLFPs for Pvu II and Xba I in the ER gene were investigated in a population of Han Chinese women to identify potential associations

Table 1. General and clinical characteristics of participants with osteoarthritis and controls by Xba I genotype ($\bar{x}\pm s$)

Genotype	XX		Xx		xx	
	OA (n=19)	Control (n=55)	OA (n=43)	Control (n=92)	OA (n=36)	Control (n=49)
Age	59.01±2.90	59.19±10.95	60.46±2.45	60.95±9.71	56.74±2.24	60.25±8.14
Height (cm)	159.85±2.43	159.72±4.79	159.70±1.56	158.57±4.55	159.72±1.20	159.30±5.18
Weight (kg)	72.50±2.75*	62.05±11.30	68.84±3.85*	58.95±8.98	70.86±2.04*	64.13±8.96
BMI (kg/m^2)	32.58±2.55*	24.21±4.90	30.59±3.50*	23.46±4.57	31.45±1.59*	25.36±3.84
BMD (g/cm^2)						
$L_{2\sim4}$	0.92±0.08	0.90±0.22	0.85±0.05	0.86±0.18	0.90±0.05	0.89±0.23
FN	0.72±0.05*	0.65±0.13	0.74±0.05*	0.67±0.13	0.78±0.04*	0.68±0.10
WT	0.47±0.06*	0.41±0.11	0.47±0.05	0.47±0.12	0.52±0.05*	0.47±0.12
TR	0.61±0.05	0.62±0.14	0.59±0.05*	0.54±0.13	0.64±0.03*	0.58±0.10

Note: *$P < 0.05$, *vs* control group. Two genotypes meet Hardy-Weinberg equilibrium ($P < 0.05$).

Table 2. General and clinical characteristics of participants with osteoarthritis and controls by Pvu II genotype ($\bar{x}\pm s$)

Genotype	PP		Pp		pp	
	OA (n=27)	Control (n=36)	OA (n=41)	Control (n=97)	OA (n=30)	Control (n=63)
Age	57.60±3.12	59.13±10.15	58.76±2.13	59.25±8.39	60.15±2.31	61.29±11.07
Height (cm)	157.95±1.93	159.22±5.19	161.30±1.59*	159.26±4.60	160.51±1.30	159.18±6.26
Weight (kg)	68.95±2.64	64.75±10.34	70.65±2.80*	60.92±9.68	71.97±3.01*	62.02±9.56
BMI (kg/m^2)	30.18±2.24	25.21±3.70	31.29±2.50*	24.14±4.67	33.37±2.64*	25.27±1.93
BMD (g/cm^2)						
$L_{2\sim4}$	0.90±0.07	0.91±0.17	0.89±0.03*	0.85±0.17	0.87±0.05	0.87±0.25
FN	0.73±0.05*	0.65±0.11	0.77±0.05*	0.68±0.11	0.72±0.04*	0.69±0.14
WT	0.49±0.06*	0.43±0.11	0.51±0.05*	0.46±0.12	0.47±0.04	0.47±0.15
TR	0.61±0.05*	0.55±0.12	0.63±0.04*	0.53±0.12	0.56±0.04*	0.63±0.14

Note: *$P < 0.05$ *vs* control group. Two genotypes meet Hardy-Weinberg equilibrium ($P < 0.05$).

with OA susceptibility and promote further understanding of OA pathogenesis

Participants and methods

Participants

Participants were recruited from Henan Province People's Hospital (Zhengzhou, Henan Province, China) between October 2011 and December 2013. Patients being treated for knee arthritis in the Department of Orthopaedics were included in the osteoarthritis group; it comprised 98 Chinese Han female patients whose mean age was 59.65±2.52 years. The diagnosis of knee arthritis was according to the diagnostic criteria revised in 2007 by Altman et al. [10]. Radiographs of knee joints were classified according to the grading scale formulated by Ravaud et al. [11], and all patients' x-rays were classified as over grade 2. Patients were treated using artificial joint replacement, joint debridement, joint fusion, and/or osteotomy. The control group comprised 196 healthy females who underwent physical examinations and whose mean age was 59.85±10.05 years. Participants in the control group were included if free of liver and kidney diseases, osteoarthritis, and endocrine system diseases.

Clinical assessment

All participants underwent radio-imaging of their knee joints while in a standing posture. Body mass and height were measured to calculate body mass index (BMI), where BMI=body mass/height^2 (kg/m^2). Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (Norland XR-36, U.S.) at lumbar vertebrae (L2-4), femoral neck (FN), Ward's triangle (WT), and trochanter (TR). BMD is ex-

pressed in g/cm^2. Peripheral blood samples were collected into EDTA-lined tubes. The DNA extract was conducted with phenol-chloroform and proteinase K (Merck). The extracted DNA was stored at -20°C until use.

Genotyping by PCR-RFLP

Polymerase chain reaction (PCR) primers were synthesized by Sunbiotech (Beijing, China). Sequences used were 5'-CTGCCACCCTATCTGTATCTTTT CCTATTCTCC-3' for the upstream primer and 5'-TCTTTCTCTGCCACCCTGG CGTCGATTATCTGA-3' for the downstream primer. PCR was performed for each sample in a volume of 50 μL, which contained 1% Triton X-100, 10 mM Trsi-HCL (pH=9.0), 2.5 mM $MgCl_2$, 50 mM KCl, 2 mM Deoxyribonucleoside triphosphates (dNTPs, Promega, Madison, USA), 10× amplification buffer, 0.25 μM each primer, Taq DNA polymerase (Promega, Madison, USA), and template DNA (300 ng). Reactions were performed in the Eppendorf Master cycler 5333 with pre-denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 40 s, and extension at 72°C; and final extension at 72°C for 10 min. A 1.3-kb amplified fragment, which included 2 exons and 1 intron, was obtained. PCR products were digested with 5 U of restriction endonuclease Xba I (Promega, Madison, USA) for 3 hours. Digested samples were separated by 1% agarose gel electrophoresis, detected by ethidium bromide, and visualized by UV Gel Imaging System Model BS60ChampGel6000.

ESR1 genotypes were determined by the presence or absence of restrictions sites. The Xba I restriction site was designated as present (x) or absent (X), with a heterozygous genotype represented as Xx. The Pvu II restriction site was designated as present (p) or absent (P), with a heterozygous individual represented as Pp. Thus, 9 different genotypes were possible: XXPP, XXPp, XXpp, XxPP, XxPp, Xxpp, xxPP, xxPp, and xxpp.

Statistical methods

SAS 9.2 was used to analyze the data by chi-squared, analysis of variance (ANOVA), and unconditional logistic regression tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Association of RLFPs of the ER gene with clinical characteristics of osteoarthritis

Clinical characteristics of participants were stratified by genotype (**Tables 1** and **2**). The mean age and body weight were similar among Xba I genotypes between the OA group and control group (each p value > 0.05). However, body mass and BMI were both significantly higher in participants with OA for each Xba I genotype than in the controls (each p value < 0.05). $L_{2\text{-}4}$ BMD did not differ significantly among the three genotypes between the two groups (each p value > 0.05). In contrast, the FN-BMD was significantly higher in the OA group than in the control group ($p < 0.05$). Similarly, the WT-BMD of individuals with genotype XX or xx in the OA group was significantly higher than their respective controls (each p value < 0.05); and the TR-BMD of individuals with genotype Xx or xx in the OA group was significantly higher than in their respective controls (each p value < 0.05).

The mean age was similar among participants with different Pvu II genotypes of the ER gene between the OA group and control group (p value > 0.05). The mean body weight of participants with genotype Pp in the OA group was significantly higher than that of the respective control group ($P < 0.05$), but body height of participants with genotype PP or pp was similar between groups (each p value > 0.05). The BMI of participants with any Pvu II genotype and the body mass of participants with genotype Pp or pp were significantly higher in the OA group than in the control group (each P value < 0.05). Further, the $L_{2\text{-}4}$ BMD of participants with genotype Pp was significantly higher in the OA group than in the control group ($P < 0.05$); the WT-BMD of participants with genotype PP or Pp was significantly higher in the OA group than in the control group (each $p < 0.05$); and the differences in TR-BMD or FN-BMD for participants with any Pvu II genotype were significantly different between groups (each p value < 0.05).

Frequency distribution of 9 genotypes of the ER gene in both groups

The distributions of the 9 possible genotypes among participants in each group were compared to determine whether any genotype(s)

Table 3. Genotype frequencies for RFLPs of the ER gene between participants with osteoarthritis and controls [n (%)]

Genotype	OA (n=98)	Control (n=196)	χ^2	*P*
PPXX	14 (14.29)	24 (12.24)	17.52	0.025
PPXx	5 (5.10)	11 (5.61)		
PpXX	2 (2.04)	28 (14.29)		
ppXX	2 (2.04)	8 (4.08)		
PpXx	27 (27.55)	49 (25.00)		
ppXx	11 (11.22)	31 (15.82)		
PPxx	6 (6.12)	5 (2.55)		
Ppxx	12 (12.24)	17 (8.67)		
ppxx	19 (19.39)	23 (11.73)		

Table 4. Osteoarthritis risk by different genotypes of *ESR1* [n, (%)]

Genotype	Control (n=196)	OA (n=98)	*OR* (95% *CI*)	*P*
PP	36 (18.37)	27 (27.55)	1.73 (0.65~3.94)	0.071
Pp	97 (49.49)	41 (41.84)	0.82 (0.45~1.51)	0.215
pp	63 (32.14)	30 (30.61)	0.89 (0.37~1.79)	0.790
XX	55 (28.06)	19 (19.39)	0.64 (0.27~1.14)	0.106
Xx	92 (46.94)	43 (43.88)	0.92 (0.32~1.73)	0.620
xx	49 (25.00)	36 (36.73)	1.98 (1.13~4.20)	0.036
PPXX	24 (12.24)	14 (14.29)	1.21 (0.49~2.97)	0.623
PPXx	11 (5.61)	5 (5.10)	0.98 (0.58~1.42)	0.856
PpXX	28 (14.29)	2 (2.04)	0.17 (0.08~0.69)	0.001
ppXX	8 (4.08)	2 (2.04)	0.51 (0.01~49.21)	0.363
PpXx	49 (25.00)	27 (27.55)	0.73 (0.35~2.13)	0.638
ppXx	31 (15.82)	11 (11.22)	0.69 (0.48~1.97)	0.289
PPxx	5 (2.55)	6 (6.12)	1.45 (0.64~3.11)	0.128
Ppxx	17 (8.67)	12 (12.24)	1.74 (0.63~4.92)	0.333
ppxx	23 (11.73)	19 (19.39)	1.37 (0.81~1.97)	0.077

Note: The height, BMI, and femur BMD are controlled.

occurred more commonly in one group (**Table 3**). The frequencies of the 9 genotypes (Xba I/Pvu II) of the ER gene were significantly different between participants with OA and controls ($P < 0.05$).

Effects of ER genotypes on the risk of occurrence of osteoarthritis

Since the distribution of ER genotypes differed between individuals with OA and controls, a statistical analysis was used to determine whether any genotype(s) conferred increased risk of OA (**Table 4**). Indeed, genotype xx was associated with increased risk of OA (*OR*=1.98, 95% *CI*: 1.13-4.20, *P*=0.036). In contrast, genotypes Pp and XX were protective against OA (*OR*=0.17, 95% *CI*: 0.08-0.69, *P*=0.001).

Discussion

Estrogen receptors specifically bind to estrogens to form estrogen-estrogen receptor complexes that are necessary for estrogens to function. The estrogen receptors located inside cytoplasm or nuclei can serve as transcription factors. Binding of ER by estrogens forms dimers that stimulate target gene transcription and promote cell proliferation and differentiation [12]. The gene encoding estrogen receptor-α, *ESR1,* is localized on chromosome 6q and includes 7 introns and 8 exons over a 140-kb span [13]. A point mutation occurring in intron 1-which also contains the enhancer elements-at the recognition sites of the Xba I and Pvu II restriction enzymes can affect the function of the ER, thereby influencing its biological actions [14]. Indeed, polymorphisms of the Xba I and Pvu II restriction sites in *ESR1* are correlated with endometriosis, uterine fibroids, breast cancer, and osteoporosis [15-17]. Further, a study of a Caucasian population demonstrated an association between polymorphisms of ER genes and hip osteoarthritis [18].

This analysis of individuals with OA compared with healthy individuals demonstrates that differences in genotypic distribution are detectable between the populations. The xx genotype was more common in individuals with OA and was associated with an almost 2-fold increased risk of developing the disorder; conversely, the genotype XX was more common in healthy controls. Further, the femoral BMD, body mass, and BMI in the osteoarthritis patients with Xba I and Pvu II as the alleles were all higher than those in the control group. Thus, genotype xx may promote increased femoral BMD and BMI, which, in turn, may lead to development of OA in Han Chinese females. These findings are consistent with those of other ethnic groups [19, 20]. Another study, which involved 1,483 older Dutch patients with OA, showed that polymorphisms of the ER gene were correlated with OA, and the

PX allele was associated with a markedly increased prevalence of radiographic knee osteoarthritis [21]. Thus, Xba I and Pvu II genotypes of *ESR1* appear to contribute to the pathogenesis of OA in a variety of populations.

In summary, this study found that the presence of two Xba I restriction sites (xx) in the *ESR1* gene is associated with increased susceptibility to OA in Han Chinese females. These results can aid in the early detection and prevention of OA, as estrogen receptor genotypes can be detected to screen for high-risk groups. The mechanism underlying the contribution of ERs to osteoarthritis remain elusive and require further study.

Disclosure of conflict of interest

None.

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