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Brief communication

Estrogen receptor alpha gene polymorphisms and breast cancer risk

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Summary

We conducted a hospital-based case-control study to evaluate the association between the *XbaI* and *PvuII* restriction fragment length polymorphisms (RFLPs) in intron 1 of the estrogen receptor α (*ER*α) gene and breast cancer risk. The study population consisted of 205 histologically confirmed incident breast cancer cases and 205 age-matched controls with no present and previous history of cancer. The *PvuII* genotype distribution did not show any difference between cases and controls, but the adjusted odds ratio (OR) for the *XbaI* X allele containing genotypes was 0.4 (95% CI: 0.3–0.6) compared with the xx genotype. The decrease in the OR appeared to be more attributable to the postmenopausal women; the ORs were 0.3 (95% CI: 0.1–0.5) and 0.5 (95% CI: 0.3–0.9) for postmenopausal and premenopausal women, respectively. Our results therefore suggest that the *ER*α *XbaI* polymorphism modifies individual susceptibility to breast cancer in Korean women.

Introduction

Breast cancer is the second most frequent cancer in Korean women and the incidence is increasing [1]. Familial history of breast cancer and reproductive factors are well-established risk factors of breast cancer. However, genetic polymorphisms of genes involved in metabolism of hormones or other carcinogens are evolving as important determinants of breast cancer susceptibility [2].

The estrogen receptor α (*ER*α) is an important mediator of the hormonal response in estrogen-sensitive tissues such as breast, endometrium, and bone. In agreement with this, potentially important polymorphisms in the *ER*α gene have, although inconsistently, been associated with bone density [3] and breast cancer [4–7] and endometrial cancer risks [8].

Most of these studies on *ER*α gene polymorphisms and breast cancer were conducted in western countries. Since oriental women may have different genotype distribution or different level of susceptibility compared with western women, we conducted a hospital-based case-control study to examine this issue further by evaluating the potential association between the genetic polymorphism of intron 1 (*PvuII* and *XbaI*) of *ER*α gene and breast cancer risk in Korean women.

Materials and methods

The study population was selected from three teaching hospitals located in Seoul, South Korea (Seoul National University Hospital, Borame Hospital and Asan Medical Center) between March 1994 and December

2000. Women with a histopathologically confirmed, incident breast cancer were selected as cases. Controls with no present and previous history of cancer were recruited in the same hospital. Most of eligible controls were being treated for gastrointestinal problems such as acute appendicitis, cholecystitis, gall bladder or bile duct stones, hemorrhoid and inguinal hernia. Women with amenorrhea, previous history of hysterectomy, oophorectomy, hormone replacement therapy, hormone-related diseases such as thyroid disease, and systematic diseases such as diabetic mellitus or chronic liver disease were excluded from both groups. Approximately 21% of cases and 6% of controls eligible were excluded from the study participants because of refusal to participate, failure to interview or blood collection.

Three hundred and six cases and 234 controls were eligible for the study. Each case frequency-matched to one control according to age groups as follows: under 29, 30–34, 35–39, 40–54, 55–69, and over 70 years. Finally, study population consisted of 205 cases and their 205 age-matched referents.

Informed consents were obtained at the time of blood sampling. Information on demographic characteristics, education, marital status, family history of breast cancer in first and second degree relatives, reproduction and menstruation, life style habits, and diet were collected by trained interviewer using a questionnaire.

Blood were collected in 10 ml heparinized tubes and DNA was isolated using standard methods. Details of *ERα PvuII* and *XbaI* restriction fragment length polymorphisms (RFLPs) analysis were described in a previous study [9]. Briefly, in the RFLPs analyses the absence and presence of *PvuII* and *XbaI* restriction sites determined the P and X, and p and x alleles, respectively.

Using standard χ^2-statistics, we tested if allele frequencies deviated from the Hardy–Weinberg equilibrium. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression model after adjustment for age, education level and family history of breast cancer. To evaluate potential interactive effects between genotypes and other risk factors, the product terms of genotypes and alcohol consumption was added in the logistic model for testing the multiplicative interaction effect of these two variables. Interactions between genotypes and reproductive factors such as age at the first full-term pregnancy, age at menache and the number of full-term pregnancy were also evaluated.

This study was conducted under approval of the Institutional Review Board of Seoul National University Hospital.

Results

The characteristics of the study populations were similar to those reported in previous studies [10]. The distributions of education, age at full-term pregnancy (FFTP), family history of breast cancer, and alcohol consumption were significantly different between cases and controls (data not shown).

The distribution of *ERα PvuII* and *XbaI* genotypes are shown in Table 1. The *PvuII* and *XbaI* genotype in controls agreed with those previously observed in Korean women [11], but were significantly different from the frequencies observed in a Swedish study [8]: where the prevalence of XX genotype in controls was about 3-fold (10.2%), and the prevalence of PP genotype 2-fold (29.9%) compared with the respective values (3.6 and 13.3%) in our study controls. Allele frequencies of *PvuII* and *XbaI* genotype of controls were not deviated from the Hardy–Weinberg equilibrium.

The *PvuII* genotype distribution did not show any difference between cases and controls, but the *XbaI* X allele containing genotypes showed decreased risk of breast cancer (OR: 0.4, 95% CI: 0.3–0.6) compared with the xx genotypes. This decrease was more attributable to postmenopausal breast cancer risk (OR: 0.3, 95% CI: 0.1–0.5). Combined with *XbaI* and *PvuII*, ORs were 2.4 (95% CI: 1.4–3.9) for xxPP or xxPp genotypes and 2.5 (95% CI: 1.5–4.0) for xxpp genotype compared with genotypes containing X allele, and their increased risks were statistically significant (p for trend <0.001).

When data were stratified by known breast cancer risk factors, nulliparous women or women with first full-term pregnancy at over 30 years of age and with xx genotype were at about 4-fold risk (OR: 4.0, 95% CI: 1.9–8.8) compared to women with X allele containing genotypes and first full-term pregnancy before 29 years (Table 2). No statistically significant multiplicative interaction, however, was observed between the *XbaI* genotypes and age at first full-term pregnancy. The xx genotype carrying drinkers who drink more than once in a week also showed an increased risk of breast cancer (OR: 3.8, 95% CI: 1.9–7.6), but no significant multiplicative interaction was observed.

Table 1. Frequency of *ERα XbaI* and *PvuII* genotypes in the study populations

Genotype		All women			Premenopausal women			Postmenopausal women		
		Cases (%) ($n = 201$)	Controls (%) ($n = 195$)	Adjusted OR (95% CI)*	Cases (%) ($n = 122$)	Controls (%) ($n = 109$)	Adjusted OR (95% CI)*	Cases (%) ($n = 79$)	Controls (%) ($n = 81$)	Adjusted OR (95% CI)*
XbaI										
xx		130 (64.7)	86 (44.1)	1.1 (0.4–2.9)	77 (63.1)	52 (47.7)	1.0	53 (67.1)	32 (39.5)	1.0
Xx		60 (29.8)	102 (52.3)	0.4 (0.2–0.6)	39 (32.0)	54 (49.5)	0.8 (0.2–3.4)	21 (26.6)	46 (56.8)	0.2 (0.1–0.4)
XX		11 (5.5)	7 (3.6)	0.9 (0.3–2.6)	6 (4.9)	3 (2.8)	0.4 (0.1–1.7)	5 (6.3)	3 (3.7)	1.1 (0.2–5.0)
xx		130 (64.7)	86 (44.1)	1.0	77 (63.1)	52 (47.7)	1.0	53 (67.1)	32 (39.5)	1.0
XX or Xx		71 (35.3)	109 (55.9)	0.4 (0.3–0.6)	45 (36.9)	57 (52.3)	0.5 (0.3–0.9)	26 (32.9)	49 (60.5)	0.3 (0.1–0.5)
PvuII										
PP		35 (17.4)	26 (13.3)	1.0	21 (17.2)	18 (16.5)	1.0	14 (17.7)	8 (9.9)	1.0
Pp		91 (45.3)	105 (53.9)	0.6 (0.4–1.2)	56 (45.9)	58 (53.2)	0.9 (0.4–1.8)	35 (44.3)	45 (55.5)	0.4 (0.2–1.2)
pp		75 (37.3)	64 (32.8)	0.9 (0.5–1.7)	45 (36.9)	33 (30.3)	1.2 (0.6–2.7)	30 (38.0)	28 (34.6)	0.7 (0.2–1.9)
PP or Pp		126 (62.7)	131 (67.2)	1.0	77 (63.1)	76 (69.7)	1.0	49 (62.0)	53 (65.4)	1.0
pp		75 (37.3)	64 (32.8)	1.3 (0.8–1.9)	45 (36.9)	33 (30.3)	1.4 (0.8–2.4)	30 (38.0)	28 (34.6)	1.3 (0.7–2.6)
Combined genotypes										
XbaI	*PvuII*									
XX, Xx	PP, Pp, pp	71 (35.4)	109 (55.8)	1.0	45 (36.9)	57 (52.2)	1.0	26 (32.9)	49 (60.4)	1.0
xx	PP, Pp	64 (31.8)	43 (22.1)	2.4 (1.4–3.9)	37 (30.3)	26 (23.9)	1.8 (0.9–3.5)	27 (34.2)	16 (19.8)	3.8 (1.7–8.8)
xx	pp	66 (32.8)	43 (22.1)	2.5 (1.5–4.0)	40 (32.8)	26 (23.9)	1.9 (1.0–3.6)	26 (32.9)	16 (19.8)	3.9 (1.7–9.1)
p for trend				<0.001			<0.05			<0.001

*OR were adjusted for age, education level and family history of breast cancer.

Table 2. Association between *XbaI* genotyes of *ERα* and risk of breast cancer stratified by selected risk factors

Stratified variables	*XbaI*		*p* for interaction
	XX, Xx	xx	
	Adjusted OR (95% CI)* (cases/controls)	Adjusted OR (95% CI)* (cases/controls)	
Alcohol consumption			
<1/month	1.0 (reference) (54/91)	2.3 (1.4–3.6) (96/71)	
≥1/month	1.6 (0.8–3.3) (17/18)	3.8 (1.9–7.6) (34/15)	0.97
Age at first full-term pregnancy (years)			
Under 29	1.0 (reference) (58/99)	2.3 (1.5–3.6) (103/75)	
Nulliparous or over 30	2.2 (0.9–5.4) (13/10)	4.0 (1.9–8.8) (26/11)	0.59

*Adjusted for age, education, and family history of breast cancer.

Discussion

Our study indicates the *ERα XbaI* genotype may modify individual breast cancer risk in Korean women, especially in postmenopausal women, whereas *PvuII* genotypes appear not to have any significant role in this context. Our findings agree with a Norwegian case-control study where women with the x allele showed a tendency of increased breast cancer risk [4], while no effect was seen for the *PvuII* genotypes. Similar findings to the Norwegian study arose in another Scandinavian (Swedish) case-control study on the association between endometrial cancer risk and *ERα* polymorphisms [8].

The most interesting finding in this study was that increased risk was observed prominently in postmenopausal women, suggesting that *XbaI* polymorphism may be associated with the breast cancer of late-onset or of onset after menopause. These findings were consistent with a previous study showing the possible linkage of the *ERα* gene polymorphism and late-onset breast cancer [12].

In a Japanese study evaluating the association between *XbaI* and *PvuII* polymorphic sites and bone mineral density in postmenopausal women, Px haplotype of *ERα* intron 1 site was related with low bone mineral density [3]. Because the *XbaI* and *PvuII* polymorphic sites are not located in the coding region of the *ERα* gene, they are not anticipated to have direct functional significance in the respective enzyme activity. However, these polymorphisms may act in several other ways. For instance, receptor function could be affected through differential splicing of mRNA [13, 14]. It is also possible that they are in linkage disequilibrium with other yet unknown regions in the gene responsible for the disease susceptibility.

A recent study suggested that the age of menache delayed in women with the XX genotype [15]. This finding is consistent with the epidemiological features of breast cancer, because the age of menache is related with cumulative lifetime estrogen exposure. Women who experienced menache before 14 years old had increased risk of breast cancer up to 2.5-folds compared with those who have their menache after 17 years old in Korean studies [16]. Another study suggested that women with the PP genotype had earlier onset of menopause than women with the pp genotype [17]. So the *ERα* polymorphisms may influence the breast cancer development *via* regulating the time of menache or menopause. But in our study, there was no difference in the age at menache or menopause between genotypes.

To conclude, our data provided support to the suggestion that *ERα* gene variants may modify individual breast cancer susceptibility. We are currently seeking for novel *ERα* gene polymorphisms. Future association studies with larger sample size and looking at these newly identified polymorphisms will further strengthen the understanding on *ERα* polymorphism and breast cancer risks.

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References

1. Central Cancer Registry Center in Korea, Ministry of Health and Welfare: Annual Report of the Central Cancer Registry in Korea (1999.1–1999.12), 2001
2. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ, Easton DFA: Systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidem Biomark Prev 8: 843–854, 1999
3. 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 11: 306–311, 1996
4. Andersen TI, Hermdal KR, Skrede M, Tveit K, Berg K, Borresen AL: Oestrogen receptor (*ESR*) polymorphism and breast cancer susceptibility. Hum Genet 94: 665–670, 1994
5. Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, Parl FF: Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 87: 446–451, 1995
6. Iwase H, Greenman JM, Barnes DM, Hodgson S, Bobrow L, Mathew CG: Sequence variants of the estrogen receptor (ER) gene found in breast cancer patients with ER negative and progesterone receptor positive tumors. Cancer Lett 108: 179–184, 1996
7. Schubert EL, Lee MK, Newman B, King MC: Single nucleotide polymorphisms (SNPs) in the estrogen receptor gene and breast cancer susceptibility. J Steroid Biochem Mol Biol 71: 21–27, 1999
8. Weiderpass E, Persson I, Melhus H, Wedrén S, Kindmark A, Baron JA: Estrogen receptor α gene polymorphisms and endometrial cancer risk. Carcinogenesis 21: 623–627, 2000
9. Nishio H, Hayashi C, Lee MJ, Ayaki H, Yamamoto R, Ninomiya R, Koizumi N, Sumino K: Itai–itai disease is not associated with polymorphisms of estrogen receptor α gene. Arch Toxicol 73: 496–498, 1999
10. Park SK, Yoo KY, Lee SJ, Kim SU, Ahn SH, Noh DY, Choe KJ, Strickland PT, Hirvonen A, Kang D: Alcohol consumption, glutathoin S-transferase M1 and T1 genetic polymorphisms and breast cancer risk. Pharmacogenetics 10: 1–9, 2000
11. Kim JG, Lim KS, Kim EK, Choi YM, Lee JY: Association of vitamin D receptor and estrogen receptor gene polymorphisms with bone mass in postmenopausal Korean women. Menopause 8: 222–228, 2001
12. Zuppan P, Hall JM, Lee MK, Ponglikitmongkol M, King MC: Possible linkage of the estrogen receptor gene to breast cancer in a family with late-onset disease. Am J Hum Genet 48: 1065–1068, 1991
13. Dotzlaw H, Alkhalaf M, Murphy LC: Characterization of estrogen receptor variant mRNA from human breast cancers. Mol Endocrinol 6: 773–785, 1992
14. Fuqua SA, Fitzgerald SD, Allred DC, Elledge RM, Nawaz Z, McDonnell DP, O'Malley BW, Greene GL, McGuire WL: Inhibition of estrogen receptor action by a naturally occurring variant in human breast tumors. Cancer Res 52: 483–486, 1992
15. Stavrou I, Zois C, Ioannidis JPA, Tsatsoulis A: Association of polymorphisms of estrogen receptor α gene with the age of menache. Hum Reproduct 17: 1101–1105, 2002
16. Yoo KY, Kang D, Park SK, Kim SU, Shin A, Yoon H, Ahn SH, Noh DY, Choe KJ: Epidemiology of breast cancer in Korea: occurrence, high-risk groups, and prevention. J Korean Med Sci 17: 1–6, 2002
17. Weel AE, Unitterlinden AG, Westendorp IC, Burger H, Schuit SC, Hofman A, Helmerhorst TJ, van Leeuwen JP, Pols HA: Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. J Clin Endocrinol Metabiol 84: 3146–3150, 1999

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