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Estrogen Receptor-1 Genetic Polymorphisms for the Risk of Premature Ovarian Failure and Early Menopause

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Abstract

Background: The aim of this study was to investigate the role of the estrogen receptor 1 (ESR1) genetic polymorphisms for early menopause that was classified as premature ovarian failure (POF) and early menopause (EM) and to examine whether the associations of ESR1 genetic variants are different for POF and EM.
Methods: We selected 100 POF cases and matched 100 EM cases and 200 normal menopause (NM) controls from the Korean Multi-Center Cohort. Among them, we restricted idiopathic POF and EM cases vs NM controls by excluding POF/EM cases with medical/surgical causes. The *Xba*I (rs9340799) and *Pvu*II (rs2234693) in the ESR1 gene were genotyped. The single-nucleotide polymorphism (SNP) and haplotype effects were analyzed by multivariate logistic regression and haplotype analysis. Also nominal polytomous logistic regression was used to find whether ESR1 genetic variants are differently associated with POF and EM.
Results: The global p values for idiopathic POF and EM were 0.08 and 0.39 (SNP-based), and <0.001 and 0.12 (haplotype-based), respectively. The *Xba*I genetic variant containing the X allele was marginally significantly associated with a reduced risk of idiopathic POF (OR = 0.6, 95% CI 0.3-1.0). The P-x haplotype and diplotypes significantly decreased the risk of idiopathic POF (OR = 0.5, 95% CI 0.2-0.9; OR = 0.4, 95% CI 0.2-0.9, respectively). In contrast from POF, the P-x haplotypes and diplotypes insignificantly increased the risk for both idiopathic EM ($p_{polytomous} = 0.009$ for P-x haplotype; $p_{polytomous} = 0.02$ for P-x diplotypes).
Conclusion: Our results suggest that the ESR1 gene including *Pvu*II and *Xba*I polymorphisms may modify the risk of idiopathic premature ovarian failure (POF) but not idiopathic early menopause (EM) risk.

Introduction

Age at menopause, as well as overall years of menstruation, has major implications for women's health. The time of menstruation cessation is an important contributing risk factor for postmenopausal diseases.[1–5] In general, menopause at an early age implies greater susceptibility to various disorders. Previous studies reported that early menopause (EM) before the age of 45 was associated with a greater risk of osteoporosis, fractures, heart disease, cancer, and all-cause mortality.[6–12] The definition of early menopause from previous studies, however, was a more inclusive definition that included both premature ovarian failure (POF) and early menopause, not exclusively POF.

POF is a disorder defined as the cessation of menstruation that occurs before the age of 40.[13] The exact mechanism of POF is unclear, but several hypotheses have been proposed, such as an abnormality of the ovary or of genetic function.[14–16] In contrast, early menopause that occurs before the age of 45 years is assumed to be due to hormonal changes rather than a genetic effect. The etiology of these related conditions is unknown, because most previous studies do not distinguish

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between POF and early menopause. Additionally, because of the broader definition of early menopause used in previous studies, the actual POF risk may be underestimated and misinterpreted. Therefore, we classified early menopause into two separate categories: POF and EM. As defined in previous studies, POF is menopause that occurs before the age of 40, and EM is defined as menopause that occurs between ages 40 and 44.[17]

Studies have investigated the association between genetic factors and age at menopause.[18–28] Genetic polymorphisms of estrogen receptors (ERs) expressed by ER-α (ESR1/estrogen receptor 1) and ER-β (ESR2/estrogen receptor 2)[29] have been examined.[21–28] The two most widely studied polymorphisms of ESR1 are *Xba*I and *Puv*II, which are located on the first intron 397 and 351 bp upstream of exon 2, and have a high degree of linkage disequilibrium.[30] Though many studies suggest that genetic polymorphisms of ESR1 modify susceptibility to women's disorders, including osteoporosis, endometriosis, preeclampsia, and breast cancer, few studies have demonstrated the association of ESR1 and the risk of POF and/or EM.

ESR1 genetic polymorphisms seem to play a role in developing POF and/or EM, but the exact mechanism is still unclear. Thus, we hypothesized that genetic variants of ESR1 underlie the association with POF and/or EM, and the effect of ESR1 in POF may differ from that of EM. In this study, we classified early menopause into two groups (POF and EM), and examined whether ESR1 genetic polymorphisms are associated with only POF or both POF and EM.

Materials and Methods

Study population

Our study population was selected from the Korean Multi-Center Cancer Cohort (KMCC), a prospective cohort of participants recruited from four urban and rural areas in Korea (Haman, Chungju, Uljin, and Youngil). The study protocol for the KMCC was approved by the institutional review boards of Seoul National University Hospital and the National Cancer Center of Korea (H0110-084-002). Detailed information about the KMCC study has been described elsewhere.[17,31] We chose four districts from Chungju, one of the local centers, and performed community surveys. To maximize the participation rate, additional telephone surveys were also performed for women who did not participate in the community survey. A total of 2,668 women between the ages of 30 and 79 were recruited; the participation rate was 70.2%. We included postmenopausal women ($n = 1{,}919$) and excluded subjects over age 70 ($n = 51$) because of recall bias and subjects with no information on their age at menopause. ($n = 132$). Menopause was defined as a period of amenorrhea greater than 12 consecutive months. We defined three menopausal groups: POF ($n = 137$) was defined as cessation of menstruation before age 40; EM was defined as menopause between the ages of 40–44 years ($n = 281$); normal menopause (NM) controls ($n = 1{,}318$) were defined as menopause between the ages of 45–60. Idiopathic POF and EM were defined as POF and EM not due to any medical causes or relevant surgical causes for cessation of menstruation. Fifty-three women from the POF group and 20 from the EM group had surgical or medical menopause related to hysterectomy, oophorectomy, cancer therapy, and various other hormonal diseases. Eighty-four idiopathic POF and 261 idiopathic EM cases were identified from our eligible population. After excluding subjects without blood samples, 103 POF and 187 EM subjects remained. We matched one POF case to one EM case and two NM controls, according to age ($\leq$60, >60 years old) and the number of years from menopause ($\leq$10, >10 year). Finally, we selected 100 POF cases, 100 EM cases, and 200 NM controls, including 46 idiopathic POF and 86 idiopathic EM cases.

Data collection

All participants signed a consent form and completed a detailed standardized interview-based questionnaire that included information on demographic characteristics, family history, medical history, reproductive factors, use of oral contraceptives, physical activity, use of agricultural chemicals, cigarette smoking, alcohol consumption, and other environmental risk factors.

Genomic DNA was prepared from whole blood samples using the genomic DNA purification kit (Core-One™ Blood Genomic DNA Isolation Kit, Seoul, Korea). PCR amplification was performed using 50 ng of genomic DNA in a 30 μl reaction volume that contained 2.5 mM dNTP 0.5 ml, 2 U of *Taq* DNA polymerase (Neurotics, Seoul, Korea), and PCR primer sets. Samples were subjected to 35 amplification cycles in GeneAmp PCR system 2700 (Applied Biosystems, Foster, CA). The PCR products were digested by *Puv*II (rs2234693) and *Xba*I (rs9340799) under conditions specified by the enzyme supplier (New England Biolabs, Beverly, MA). The PCR primers were sense strand5′-ctgccaccctatctgatatcttttcctattctcc-3′ and antisense strand 5′-tctttctctgccaccctggcgtcgattatctga-3′. Restriction fragments were separated by agarose gel electrophoresis and ethidium bromide staining. The presence of the restriction site for each endonuclease was conventionally indicated with a lowercase letter (p or x, respectively, for *Puv*II and *Xba*I endonucleases), whereas the absence of the restriction site was indicated with a capital letter (P or X).

Statistical analysis

Multiple logistic regression analysis was used to estimate odds ratio (OR) and associated 95% confidence interval (CI) for environmental factors. We conducted univariate analysis to identify significant covariates from the total study population that included medical and surgical cases. Age, education, number of years from menopause to enrollment time, past history of pulmonary tuberculosis, cancer history, hysterectomy, use of oral contraceptives, age at menarche, spontaneous abortion at first pregnancy (meaning whether a women's first pregnancy was successful or failed), and duration of breast feeding were selected as covariates in our analysis. In the analysis of idiopathic cases and controls, surgical or medical menopause-related variables such as hysterectomy, cancer history, and past history of pulmonary tuberculosis were excluded as adjustment variables.

The Hardy-Weinberg equilibrium assessed allele frequencies using the χ-square test. Among POF cases, EM cases, and controls, genotype frequencies did not deviate from the Hardy-Weinberg equilibrium ($p > 0.1$). Moreover, single SNPs, haplotype, and diplotype analyses were performed. To detect each SNP effect of ESR1 for POF and EM, three genetic models—a codominant, dominant, and recessive model—

were used, and the *p*-trend values derived from the additive model were presented. Tests for significance were computed after adjusting for age, education, number of years from menopause (<10, >10 year), smoking status, past history of pulmonary tuberculosis, age at menarche, spontaneous abortion at first pregnancy, and breastfeeding; all were selected from backward stepwise logistic regression.

Risks for POF and EM in relation to ESR1 haplotypes were assessed using SAS-Genetics software (version 8.2),[32] which employs the expectation-maximization algorithm to estimate haplotypes. The *Xba*I and *Pvu*II polymorphisms were in high linkage disequilibrium (Lewontin's D' value = 0.99). A haplotype-based global score test assessed the overall differences in haplotype frequencies between POF or EM cases and NM controls. We assessed the haplotype-associated risks using the P-X haplotype as the referent category after adjusting for age, education, number of years from menopause (≤10, >10 year), smoking status, past history of pulmonary tuberculosis, age at menarche, spontaneous abortion at first pregnancy, and breastfeeding. The association between ESR1 haplotypes with observed frequencies greater than 5% and POF and EM cases was evaluated. Diplotypes with at least one copy of the referent haplotype P-X were selected as the reference in the diplotype analysis. Risk of POF or EM was estimated for each diplotype compared to the referent diplotype, adjusting for the same covariates. The diplotype data were treated as categorical variables and were incorporated as dummy variables in the logistic regression models.

Polytomous logistic regression was used to estimate *p* values for an association between genetic factors and the three menopausal groups (POF, EM, and NM) according to a nominal scale. Likelihood ratio tests for linear trend assessed a potential dose-response relationship. If any of the cell frequencies in a given table were less than three, we did not present the ORs and 95% CIs.

Results

Compared to NM controls, EM cases were more educated and POF cases were less educated ($p = 0.01$) (data not shown). Table 1 presents the odds ratios and 95% CIs of environmental and reproductive risk factors such as smoking/alcohol status, physical activity, past history of pulmonary tuberculosis, age at menarche, parity, spontaneous abortion at first pregnancy, and breast feeding, for POF and EM. Past history of pulmonary tuberculosis and age at menarche were significant risk factors for both POF and EM (OR = 1.6, 95% CI 1.5-11.0 for EM; OR = 5.0, 95% CI 1.5-16.4 for POF in terms of past history of pulmonary tuberculosis; OR = 4.1, 95% CI 1.5-11.0 for EM; OR = 5.6, 95% CI 2.0-15.4 for POF in terms of age at menarche), but an increased risk was observed in POF cases. Breastfeeding for longer than 24 months was a significant protective factor for both POF and EM (OR = 0.3, 95% CI 0.1-0.99 for EM; OR = 0.2, 95% CI 0.1-0.6 for POF). For idiopathic cases, age at menarche was significant for both EM and POF (OR = 4.8, 95% CI 1.7-14.0 for EM; OR = 7.0, 95% CI 2.2-23.0 for POF).

Table 2 shows the distribution of ESR1 polymorphisms among all cases and controls and the ORs (95% CIs) for POF and EM cases compared to NM controls in relation to ESR1 genetic polymorphisms. Genotype frequencies for all SNPs did not deviate from the Hardy-Weinberg equilibrium ($p > 0.1$). In the single SNPs analysis, no significant allele effect was observed with the *Pvu*II genotype. In contrast, The *Xba*I genetic variant containing the X allele was associated with a reduced risk for both total POF and idiopathic POF in the dominant model (OR = 0.6, 95% CI 0.3-0.99 for total POF; OR = 0.6, 95% CI 0.3-1.0 for idiopathic POF). However, this X allele effect was not observed for both total EM and idiopathic EM cases.

In the polytomous logistic regression, the dominant model showed that the *Xba*I genetic variant for POF risk was different from EM, relative to NM controls, and showed marginal significance ($p_{polytomous} = 0.08$). The *Xba*I genetic variant for idiopathic POF risk was also different from idiopathic EM ($p_{polytomous} = 0.08$). The two SNP-based global *p* values were 0.17 for EM cases vs controls and 0.07 for POF cases vs controls for all cases including medical/surgical causes; and 0.39 for EM cases vs controls and 0.09 for POF cases vs controls for idiopathic causes.

Table 3 shows the association between haplotype-pairs of the ESR1 gene and POF and EM risk. In the haplotype analysis, three *Pvu*II- *Xba*I haplotypes, P-X, P-x and p-X, were observed. The frequency of the p-x haplotype was less than 2% in each group. The haplotype-based global *p* value was 0.06 for EM cases vs controls and <0.001 for POF cases vs controls for all cases including medical/surgical causes; and 0.12 for EM cases vs controls and <0.0001 for POF cases vs controls for idiopathic causes.

Compared to the most frequent P-X haplotype, the P-x haplotype significantly decreased the risk of both total POF and idiopathic POF (OR = 0.5, 95% CI 0.3-0.7 for total POF; OR = 0.5, 95% CI 0.2-0.9 for idiopathic POF). The p-X haplotype also showed a decreased risk for both total POF and idiopathic POF but was not significant. In contrast to POF, the P-x and p-X haplotypes increased the risk for both EM and idiopathic EM but was not statistically significant. The P-x and p-X haplotype effects were different for POF and EM and idiopathic POF and idiopathic EM. All were significant by polytomous regression analysis except the p-X haplotype for idiopathic POF and EM ($p_{polytomous} = 0.0001$, $p_{polytomous} = 0.009$, $p_{polytomous} = 0.046$, respectively).

We recombined the six diplotypes into three groups and set the P-X*P-X or P-X*p-X diplotypes as the reference group. Compared to the reference group, diplotypes that contained one or two copies of P-x statistically significantly decreased the risk of both total POF and idiopathic POF (OR = 0.4, 95% CI 0.2–0.6 for total POF; OR = 0.4, 95% CI 0.2-0.9 for idiopathic POF). Also, diplotypes with one or two copies of p-X except P-X*p-X decreased the POF risk regardless of idiopathic type but were insignificant. One or two copies of the P-x diplotype was significantly different from POF and EM risk, regardless of idiopathic type ($p_{polytomous} = 0.0001$, $p_{polytomous} = 0.02$) (Table 3).

Discussion

Our study showed that the ESR1 genetic variant was significantly associated with POF risk but not with EM. The X allele of *Xba*I and specific haplo- and diplotype of *Pvu*II and *Xba*I polymorphisms were associated with a significantly reduced risk of POF occurrence but not EM. This association remained in stratified analysis of only idiopathic cases. Moreover, results of nominal polytomous logistic regression

Table 1. The Association with Exposure to Environmental and Reproductive Risk Factors Before Menopause in Three Menopausal Groups

	Total cases including surgical and medical causes vs *controls*							*Idiopathic cases* vs *controls*			
	NM controls No.	*EM cases No.*	*OR (95% CI)*[a]	*POF cases No.*	*OR (95% CI)*[a]	p-*polytomous*[c]	*Idiopathic EM cases No.*	*OR (95% CI)*[b]	*Idiopathic POF cases No.*	*OR (95% CI)*[b]	p-*polytomous*[c]
Total number	200	100		100			86		46		
Cigarette smoking[e] (Ever)	29	18	1.3 (0.6-2.5)	24	1.2 (0.6-2.7)	0.70	15	1.2 (0.5-2.7)	14	1.6 (0.6-4.0)	0.11
Environmental smoking[f] (Ever)	172	84	0.7 (0.3-1.4)	84	1.0 (0.4-2.4)	0.90	74	0.8 (0.3-1.9)	50	0.8 (0.3-2.3)	0.22
Alcohol consumption[e] (Ever)	55	25	0.7 (0.4-1.2)	27	0.6 (0.3-1.1)	0.25	17	0.5 (0.2-1.3)	14	1.5 (0.5-4.0)	0.45
Physical activity per week[g] (Intense)	71	25	0.9 (0.4-1.8)	42	1.4 (0.6-3.2)	0.12	25	1.0 (0.5-2.2)	21	0.7 (0.3-1.9)	0.98
Sedentary time per day (≥7 hours)	32	18	1.0 (0.5-1.9)	20	1.4 (0.7-2.8)	0.72	16	1.1 (0.5-2.4)	14	0.4 (0.1-1.5)	0.17
Use of agricultural chemicals (Yes)	27	14	0.9 (0.4-2.0)	12	1.0 (0.4-2.4)	0.90	14	1.7 (0.3-6.9)	9	1.7 (0.6-4.4)	0.98
Past history of pulmonary tuberculosis[h] (Yes)	6	4	1.6 (1.5-11.0)*	8	5.0 (1.5-16.4)*	0.05	–	–	–	–	
Hysterectomy (Yes)	13	13	1.2 (0.5-3.1)	43	9.1 (3.8-21.8)**	<0.01					
Cancer (Yes)	2	6	6.2 (1.1-34.0)*	2	–[d]	0.22					
Age at menarche (≤13 yr)	8	14	4.1 (1.5-11.0)**	22	5.6 (2.0-15.4)**	0.73	11	4.8 (1.7-14.0)*	10	7.0 (2.2-23.0)*	0.31
Menstruation regularity[i] (Regular)	171	89	2.0 (0.8-5.5)	82	1.2 (0.5-2.7)	0.14	76	1.7 (0.6-5.2)	43	–[j]	0.97
Parity[k] (≥4)	147	63	0.8 (0.5-1.5)	51	0.8 (0.4-1.7)	0.67	58	0.9 (0.5-1.6)	35	1.0 (0.6-1.8)	0.16
Use of oral contraceptives (Yes)	65	22	0.6 (0.3-0.9)*	32	0.7 (0.3-1.3)	0.22	22	0.4 (0.2-0.8)**	18	1.1 (0.5-2.5)	0.02
Spontaneous abortion at first pregnancy[l] (Yes)	6	15	6.0 (2.1-16.8)**	3	–[d]	0.02	9	3.7 (1.1-13.1)*	3	–[d]	0.49
Artificial abortion in the first pregnancy[l] (Yes)	2	–		9	4.2 (0.7-26.6)	0.97	0		3	–[d]	–
Experience of breast feeding[l] (Yes)	197	95	0.2 (0.0-1.0)†	93	0.3 (0.1-1.3)	0.73	83	0.2 (0.1-1.0)†	54	0.6 (0.1-4.1)	0.43
Duration of breast feeding[l] (≥24mo)	28	5	0.3 (0.1-0.99)*	8	0.2 (0.1-0.6)*	0.17	5	0.4 (0.1-1.2)	8	0.3 (0.1-1.5)	0.27

*$p < 0.05$, **$p < 0.01$, †$0.05 \leq p < 0.1$.

[a]Odds ratios and 95% confidence intervals adjusted for age, education, number of years from menopause to enrollment time, past history of pulmonary tuberculosis, cancer history, hysterectomy, use of oral contraceptives, age at menarche, spontaneous abortion at first pregnancy, and duration of breast feeding.

[b]Odds ratios and 95% confidence intervals adjusted for age, education, number of years from menopause to enrollment time, use of oral contraceptives, age at menarche, spontaneous abortion at first pregnancy, and duration of breast feeding.

[c]*p* value by the polytomous logistic regression model of nominal scale.

[d]If the sample size in a cell for analysis was three or fewer, we did not calculate the ORs (95% CIs).

[e]Defined as women who smoked cigarettes or consumed alcohol at least one year before menopause.

[f]Environmental smoking was defined as women who lived in the same house for at least one year prior to menopause with a family member who smoked at least 20 packs of cigarettes in a lifetime.

[g]Physical activity per week was classified into two groups (never/mild/moderate vs intense physical activity). The definitions were as follows: Intense, intense labor or physical exercise at least 21 hours per week (bicycling, playing tennis, swimming, etc.); Moderate, intense labor or physical exercise for 1–20 hours per week or moderate labor or exercise more than 4 hours per week (walking, playing golf, bicycling, dancing, gardening, cleaning and washing at home, etc.); Mild, moderate activity fewer than 3 hours per week; Never, physical exercise or labor under 20 minutes per week.

[h]Past lung tuberculosis was defined by self-reported tuberculosis history and chest X-ray.

[i]Regular menstruation cycle was defined as menstruation occurring every 21 to 35 days.

[j]Among POF cases, there were no women with regular menstruation.

[k]Defined as women who derived more than 4 children or less than 4 children (1–3) among parous women; all subjects were parous women.

[l]Among parous women; all subjects were parous women; total number responding to "Duration between 0 to 24 months of breast feeding" for normal controls, EM, and POF subjects were 13, 18, 37, respectively.

NM, normal menopause; EM, early menopause; OR, odds ratio; POF, premature ovarian failure; CI, confidence interval.

Table 2. SNP Effect of the Genetic Polymorphisms of ESR1 (*Pvu*II and *Xba*I) for POF or EM

	Total cases including surgical and medical causes vs. controls						*Idiopathic cases vs controls*					
	NM controls[a] *No.*	*EM cases*[a] *No.*	*OR (95% CI)*[b]	*POF cases*[a] *No.*	*OR (95% CI)*[b]	p-*polytomous*[c]	*Idiopathic EM cases*[a] *No.*	*OR (95% CI)*[b]	*Idiopathic POF cases*[a] *No.*	*OR (95% CI)*	p-*polytomous*[c]
Total number	200	100		100			86		46		
*Pvu*II genotypes											
Codominant model[d]											
p/p	66	27	1.0 (reference)	29	1.0 (reference)		27	1.0 (reference)	18	1.0 (reference)	
P/p	106	52	1.2 (0.7-2.2)	54	1.2 (0.6-2.1)	0.92	45	1.1 (0.6-2.1)	23	0.9 (0.4-2.1)	0.99
P/P	28	21	2.0 (0.9-4.7)	17	1.5 (0.6-3.7)	0.51	14	1.6 (0.7-3.9)	5	1.1 (0.3-3.9)	0.71
p-trend			0.12		0.37			0.34		0.98	
Dominant model											
p/p	66	27	1.0 (reference)	29	1.0 (reference)		27	1.0 (reference)	18	1.0 (reference)	
P/p and P/P	134	73	1.3 (0.7-2.4)	71	1.2 (0.7-2.2)	0.92	59	1.2 (0.7-2.2)	28	1.0 (0.5-2.1)	0.99
Recessive model											
p/p & P/p	172	79	1.0 (reference)	83	1.0 (reference)		72	1.0 (reference)	41	1.0 (reference)	
P/P	28	21	1.7 (0.9-3.5)	17	1.4 (0.6-2.9)	0.46	14	1.5 (0.7-3.2)	5	1.1 (0.4-3.7)	0.68
*Xba*I genotypes											
Codominant model[d]											
x/x	90	48	1.0 (reference)	58	1.0 (reference)	–	36	1.0 (reference)	24	1.0 (reference)	
X/x	102	47	0.9 (0.5-1.5)	38	0.6 (0.3-0.96)*	0.08	45	1.1 (0.6-1.8)	21	0.6 (0.3-1.3)	0.11
X/X	8	5	1.5 (0.4-5.0)	4	0.9 (0.3-3.3)	0.42	5	1.8 (0.5-6.1)	1	–	0.16
p trend			0.97		0.10			0.56		0.14	
Dominant model											
x/x	90	48	1.0 (reference)	58	1.0 (reference)		36	1.0 (reference)	24	1.0 (reference)	
X/x and X/X	110	52	0.9 (0.5-1.5)	42	0.6 (0.3-0.99)*	0.08	50	1.1 (0.6-1.9)	22	0.6 (0.3-1.0)[†]	0.08
Recessive model											
x/x and X/x	192	95	1.0 (reference)	96	1.0 (reference)		81	1.0 (reference)	45	1.0 (reference)	
X/X	8	5	1.6 (0.5-5.2)	4	1.2 (0.4-4.3)	0.77	5	1.7 (0.5-5.7)	1	–	0.29

The two SNP-based global p value was 0.17 for EM cases vs controls and 0.07 for POF cases vs controls for all cases including medical/surgical causes; and 0.39 for EM cases vs controls and 0.08 for POF cases vs controls for idiopathic causes.

*$p < 0.05$, **$p < 0.01$, [†]$0.05 \leq p < 0.1$.

[a]Matched by age (≤60, >60 years old).

[b]Odds ratios and 95% CIs were adjusted for age, education, number of years from menopause (≤10, >10 year), smoking status, past history of pulmonary tuberculosis, age at menarche, spontaneous abortion at first pregnancy, and breastfeeding.

[c]Polytomous logistic regression by the outcome of nominal scale.

[d]No deviation from the Hardy-Weinberg equilibrium ($p > 0.1$).

Table 3. Haplotype and Diplotype Effects of ESR1 Genetic Polymorphisms (*Pvu*II and *Xba*I) for POF or EM

	Total cases including surgical and medical causes vs controls						*Idiopathic cases vs controls*				
	NM controls[a] *No.*	*EM cases*[a] *No.*	*OR (95% CI)*[b]	*POF cases*[a] *No.*	*OR (95% CI)*[b]	p-*polytomous*[c]	*Idiopathic EM cases*[a] *No.*	*OR (95% CI)*[b]	*Idiopathic POF cases*[a] *No.*	*OR (95% CI)*[b]	p-*polytomous*[c]
Total number	200	100		100			86		46		
PvuII-XbaI Haplotypes											
P-X	120	51	1.0 (reference)	78	1.0 (reference)		46	1.0 (reference)	40	1.0 (reference)	
P-x	118	55	1.1 (0.7-1.8)	34	0.5 (0.3-0.7)**	0.001	71	1.2 (0.7-2.0)	19	0.5 (0.2-0.9)*	0.009
p-X	162	92	1.4 (0.9-2.1)	76	0.8 (0.5-1.2)	0.046	53	1.3 (0.8-2.0)	29	0.7 (0.4-1.2)	0.16
PvuII-XbaI diplotypes											
One copy or two copies of P-X	62	29	1.0 (reference)	51	1.0 (reference)		24	1.0 (reference)	22	1.0 (reference)	
All diplotypes with P-x	110	50	1.0 (0.5-1.8)	32	0.4 (0.2-0.6)**	0.001	14	1.1 (0.6-2.0)	19	0.4 (0.2-0.9)*	0.02
The others	28	21	1.7 (0.8-3.7)	17	0.9 (0.4-2.0)	0.17	48	1.6 (0.7-3.6)	5	0.7 (0.2-2.4)	0.39

The haplotype-based global p value was 0.06 for EM cases *vs* controls and <0.001 for POF cases *vs* controls for all cases including medical/surgical causes; and 0.12 for EM cases *vs* controls and <0.0001 for POF cases *vs* controls for idiopathic causes.

*$p < 0.05$, **$p < 0.01$.

[a]Matched by age (≤60, >60 years old).

[b]Odds ratios and 95% CIs were adjusted for age, education, number of years from menopause (≤10, >10 year), smoking status, past history of pulmonary tuberculosis, age at menarche, spontaneous abortion at first pregnancy, and breastfeeding.

[c]Polytomous logistic regression by the outcome of nominal scale.

indicated that ESR1 genetic variants influence the etiology of POF and EM differently.

Previous studies that focused on the association of ESR1 *Pvu*II or/and *Xba*I polymorphisms and the onset of menopause did not find a significant association.[33–36] However, two studies reported that the ESR1 gene was significantly associated with the earlier onset of menopause.[22,28] In one study, the homozygous P allele of *Pvu*II was associated with 1.1 year earlier onset of menopause compared to the homozygous p allele. Also, an additive effect for each copy of the P allele was reported.[22] Similarly, in our study, the homozygous P allele of *Pvu*II was associated with an increased risk for both POF and EM in the SNP effect, although statistically insignificant, possibly because of the small sample size. In another study, one of the ESR1 haplotypes, corresponding to the P-X haplotype, was associated with a significantly increased risk for POF.[28] Concordant with this study, when the reference value was the P-x haplotype, the P-X haplotype was associated with a significantly increased risk for POF (OR = 2.2, 95% CI 1.4-3.7 for total POF; OR = 2.2, 95% CI 1.2-4.2 for idiopathic POF).

Few studies have focused on ESR1 *Xba*I polymorphisms associated with age at menopause or POF. However, the X allele of *Xba*I was reported to be related to increased bone mineral density, reduced risk of osteoporosis, and cardiovascular diseases, which suggest higher levels of estrogen.[24,37–40] In terms of our study outcome, we can also infer that the X allele of *Xba*I may play a crucial role in protection against POF. However, we did not find evidence that the X allele of *Xba*I is involved in EM development. Our study results, including our nominal polytomous logistic regression, suggest that development of POF and EM may be related to different genetic functions.

The biological pathway of *Xba*I and *Pvu*II that relates to early onset of menopause is still unknown. An *in vitro* study reported that the P allele of *Pvu*II may play a role in the amplification of ER-α transcription or may regulate the ESR1 expression and function.[41] Although there is no information on the role of the *Xba*I X allele, the *Xba*I X allele could act with the *Pvu*II P allele in ER-α transcription or ESR1 expression/function, since *Xba*I and *Pvu*II polymorphisms are in high linkage disequilibrium.

When considering previous reports and the present study results, we hypothesize a series of mechanisms for POF and ER genetic polymorphisms: (1) Estrogen binds to ERs in reproductive tissues, such as the ovaries, uterus, and vagina.[42] If the activity of ERs is low because of the low activity gene encoding protein, estrogenic action in the tissue may be weak; (2) Continuous weak estrogenic effect in the reproductive tissue, especially the ovaries, may have a negative feedback on the pituitary gland, especially follicle stimulating hormone (FSH) secretion; (3) FSH, in turn, can accelerate the rapid depletion of the ovarian follicles, leading to the development of POF because of ovarian dysfunction. To clarify the exact mechanisms between ER genes and POF and/or EM, additional studies are required.

Although, to our knowledge, this is the first study to report a genetic difference related to age at menopause, our study had several limitations. First, because of the high cost and low access to clinics and physicians in community population-based survey settings, our POF cases were ascertained only by self-report, without FSH testing or other confirmation. Thus,

we were not able to differentiate POF and premature menopause (PM) cases in our analysis.[43] Second, our study included a small number of idiopathic POF and EM cases, so we did not have sufficient statistical power to observe a gene-gene or gene-environment interaction. Third, we genotyped only a small number of SNPs related to ERs, and thus we were not able to examine other important SNPs. Finally, we classified menopausal groups based on self-reported age at menopause, which is vulnerable to misclassification and recall bias. However, in order to minimize bias, we excluded subjects over the age of 60 and adjusted for the number of years from menopause.

In conclusion, our study shows that the ESR1 gene including *Pvu*II and *Xba*I polymorphisms can modify the risk of idiopathic POF, but these variants are not associated with EM risk. This supports the possibility that the etiology of POF and EM may differ with regard to genetic factors. Further studies with sufficient POF and EM cases will help clarify the ESR1 genetic mechanism and examine the gene-gene and gene-environment interactions.

Disclosure Statement

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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