Estrogen Receptor Polymorphism Predicts the Onset of Natural and Surgical Menopause*

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ABSTRACT

Age at menopause and risk of hysterectomy have strong genetic components, but the genes involved remain ill defined. We investigated whether genetic variation at the estrogen receptor (ER) gene contributes to the variability in the onset of menopause in 900 postmenopausal women, aged 55–80 yr, of the Rotterdam Study, a population-based cohort study in The Netherlands. Gynecological information was obtained, and if women reported surgical menopause, validation of type and indication of surgery was accomplished by checking medical records. The ER genotypes (PP, Pp, and pp) were assessed by PCR using the *Pvu*II endonuclease.

Compared with women carrying the pp genotype, homozygous PP

women had a 1.1-yr ($P < 0.02$) earlier onset of menopause. Furthermore, an allele dose effect was observed, corresponding to a 0.5-yr ($P < 0.02$) earlier onset of menopause per copy of the P allele. The risk of surgical menopause was 2.4 (95% confidence interval, 1.5–3.8) times higher for women carrying the PP genotype compared to those in the pp group, with the most prominent effect in women who underwent hysterectomy due to fibroids or menorrhagia.

We conclude that genetic variations of the ER gene are related to the onset of natural menopause and the risk of surgical menopause, especially hysterectomy. (*J Clin Endocrinol Metab* **84:** 3146–3150, 1999)

PREMATURE exposure to low estrogen levels, as occurs during the early onset of menopause, has major implications for the health of postmenopausal women. An early onset of menopause is associated with a higher risk of cardiovascular diseases, osteoporosis, and ovarian cancer, and moreover, it increases the risk of mortality (1–4). Therefore, from a clinical point of view, it is important to identify factors that influence the age at menopause. Although several environmental factors have been proposed as risk factors for the early onset of menopause (5–9), genetic factors have recently been proposed to be determinants of age at menopause (10, 11). This idea is strongly supported by a recent twin study that showed that the onset of menopause is genetically determined, yielding heritability for age at menopause of 63% (12). In addition, undergoing hysterectomy before reaching natural menopause, with menorrhagia or fibroids as main indications, showed considerable heritability (59%) in the same study.

Several approaches can be followed to identify genes that might contribute to the variation in the onset of menopause, including the analysis of candidate genes. In the estrogen endocrine system the estrogen receptor (ER) is an important

candidate in this respect. This member of the family of steroid transcription factors functions as a regulator of the expression of many genes and proteins (13, 14), and furthermore, the ER is an important regulator of growth and differentiation in many tissues, including the endometrium (15–18).

The aim of the present study was to identify a genetic determinant of the onset of menopause. We investigated the association between an anonymous intronic *Pvu*II restriction fragment length polymorphism (RFLP) of the ER gene and both the natural and surgical onsets of menopause in a population-based sample of postmenopausal women.

Materials and Methods

All postmenopausal women included in this study were part of a population-based cohort study (n = 7983; 61.1% women) of persons aged 55 yr and over, living in a district of Rotterdam, The Netherlands. The objective of the study was to investigate the occurrence of chronic disabling diseases in relation to several potential determinants. Rationale and design have been described previously (19). A total of 10,275 persons, of whom 9,161 (89%) were living independently, were invited to participate in the study in 1990. Among those living independently, the overall response rate was 77% for the home interview and 71% for examination at the research center, where anthropometric characteristics and blood samples were taken. The Rotterdam Study was approved by the medical ethics committee of Erasmus University Medical School, and written informed consent was obtained from each subject.

For the present study we included independently living subjects who were initially part of a large epidemiological study of osteoporosis in which subjects according to the following criteria were excluded: aged 80 yr and over, use of thyroid hormone, use of cytostatics, use of diuretics, and known diabetes mellitus type II. Among the 4478 remaining

independently living subjects, an age-stratified sample of 1000 women was drawn with balanced numbers (n = 200) in 5-yr age categories. DNA samples and menopause data for 900 postmenopausal women were available for the analysis.

During the home interview each woman provided information on her reproductive and gynecological history, including ever use of sex steroids. Natural menopause was defined as menopausal after 12 continuous months of amenorrhea, without gynecological surgery or other procedures that would have stopped menses. In case a gynecological surgical procedure before natural menopause was reported, we validated the date and indication of surgery by checking the medical records of the general practitioner (98% of cases). The health care system in The Netherlands permits this validation, as every individual has her own general practitioner. The general practitioner is the only access to specialist and hospital care and preserves all physician and hospital notes. Age at menopause was defined as age at natural menopause or age at surgical menopause (defined as the age at the date of operation). Surgical procedures were defined as hysterectomy (women with only hysterectomy and women with hysterectomy plus uni- or bilateral oophorectomy), oophorectomy (women with only uni- or bilateral oophorectomy), and unknown type of gynecological surgery. Smoking habits (smoking defined as ever *vs.* never smoked) and socio-economic status (defined as the highest education level attained; class I–II primary school with/without lower secondary school *vs.* class III–IV secondary school with/without higher vocational school or university) were assessed by questionnaire. Height and weight were measured at the clinical examination, with the subject in a standing position without shoes.

Genotyping

The anonymous *Pvu*II RFLP is located in intron 1, 0.4 kb upstream of exon 2 of the ER gene and was assessed by a PCR procedure (20). Briefly, genomic DNA (100 ng) was extracted from peripheral leukocytes and used for PCR amplification in a reaction mixture containing 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl$_2$, 0.2 mmol/L deoxy-NTP, 150 ng of each primer, and 0.2 U Super *Taq* polymerase (HT Biotechnology Ltd., Cambridge, UK). The reactions were performed in a DNA thermocycler (mode 480, Perkin Elmer Corp., Foster City, CA) with a cycling protocol of 94, 60, and 72 C for 1 min each for 25 cycles. Ten microliters of PCR products were digested with a *Pvu*II restriction enzyme (Life Technologies, Inc., Breda, The Netherlands) and 2.5 μL of a buffer [containing 150 mmol/L Tris-HCl (pH 7.5), 250 mmol/L NaCl, and 35 mm MgCl$_2$] by incubation for 30 min at 37 C. The digestion products were analyzed by 1.4% agarose gel electrophoresis in 0.5 × TBE

(1 × TBE = 89 mmol/L Tris, 89 mmol/L boric acid, and 2 mmol/L Na$_2$ ethylenediamine tetraacetate) for 250 volt hours. Separation patterns were documented by Polaroid photography under UV illumination (302 nm). Genotypes were defined as PP, Pp, or pp. Capital letters denote the absence and lowercase letters the presence of the site for the restriction enzyme *Pvu*II (P/p). To confirm the accuracy of the genotyping, repeated analysis was performed on 100 randomly selected samples. No discrepancies were found.

Statistical analysis

One-way ANOVA and χ^2 analysis were used to compare anthropometric and environmental variables among the three genotype groups. To account for potential confounders, such as age of menarche, mean number of offspring, smoking, body mass index, socio-economic status, hormone replacement therapy, and use of oral contraceptives, we used multivariate regression models. Subsequently, we calculated the odds ratio [with 95% confidence interval (CI)] as a measure of the relative risk for occurrence and indication of surgical menopause associated with ER genotype using logistic regression models where women without premenopausal gynecological surgery were the reference group. To visualize the genetic influences on the lifetime risk of hysterectomy, we constructed a cumulative hazard function using the Cox proportional hazard regression model where women without surgery were the reference group.

Results

The allele frequencies did not deviate from the Hardy-Weinberg equilibrium, which indicates that no selection has occurred among genotypes. Table 1 shows the general characteristics of the postmenopausal women according to their *Pvu*II genotype. The three genotypes did not differ significantly in age at menarche, use of sex steroids (hormone replacement therapy and oral contraceptives), smoking, body mass index, and socio-economic status. Furthermore, the mean number of offspring and the percentage of women with children did not differ between the three genotypes. Unfortunately, we did not have data on the total number of pregnancies, which gives a better insight into fertility.

However, there was a significant difference in the mean

TABLE 1. Characteristics of 900 women according to their ER *Pvu*II genotype

| | ER *Pvu*II genotype | | | P value | |
	PP	Pp	pp	ANOVA	PP *vs.* pp
No. (%)	205 (23)	435 (48)	260 (29)		
Age (yr)	67.9 ± 7.0	67.5 ± 6.9	67.1 ± 7.1	0.4	0.2
Ever use HRT (%)	32 (15.6)	62 (14.2)	37 (14.2)	0.9	0.7
Ever use oral contraceptive (%)	52 (25.4)	106 (24.4)	61 (23.5)	0.9	0.8
Ht (cm)	162.5 ± 5.8	161.5 ± 6.3	161.7 ± 7.3	0.2	0.2
Wt (kg)	68.6 ± 10.9	68.4 ± 10.0	68.2 ± 10.6	0.9	0.5
BMI (kg/m^2)	26.0 ± 3.8	26.2 ± 3.5	26.1 ± 4.1	0.7	0.8
Ever smoked (%)	104 (50.7)	228 (52.5)	135 (52.1)	0.9	0.8
SES (education level I–II) (%)	112 (54.6)	258 (59.4)	164 (63.3)	0.2	0.1
No. of offspring	2.2 ± 1.6	2.1 ± 1.6	2.1 ± 1.8	0.6	0.4
Offspring					
None (%)	34 (16.6)	85 (19.5)	55 (21.2)		
1 or 2 (%)	93 (45.4)	198 (45.5)	116 (44.6)	0.8	0.4
≥2 (%)	78 (38.0)	152 (34.9)	89 (34.2)		
Age at menopause (yr)	48.1 ± 5.0	48.7 ± 5.0	49.2 ± 4.6	0.06	0.02
Median	49	49	50		
Age at natural menopause (yr)[a]	48.7 ± 4.8	49.4 ± 4.3	49.8 ± 4.2	0.09	0.03
Age at surgical menopause (yr)[b]	46.1 ± 5.5	44.9 ± 6.3	46.4 ± 4.7	0.4	0.8
Age at menarche (yr)	13.8 ± 1.7	13.7 ± 1.6	13.6 ± 1.7	0.4	0.3
Median	14	14	13		

[a] Natural menopause defined as onset of menopause without hysterectomy, oophorectomy, or any other procedure that stopped menses.
[b] Age at surgical menopause defined as age at date of hysterectomy and/or oophorectomy.

age at onset of menopause among the genotypes. The mean and median age at menopause were 1.1 yr ($P < 0.02$) earlier in women with the PP genotype compared to those in the pp genotype group. Furthermore, an allele-dose effect was observed, corresponding to a 0.5-yr ($P < 0.02$) earlier onset of menopause per copy of the P allele. After adjustment for the potential confounders (age at menarche, mean number of offspring, use of sex steroids (HRT and oral contraceptives), smoking, body mass index, and socio-economic status), similar findings were observed.

After excluding women with artificial menopause, the result remained essentially the same, with an earlier onset of natural menopause for the women with the PP genotype. However, the frequency of women with surgical menopause differed among the three genotypes (Table 2). Therefore, we compared the risk of surgical menopause for women by their ER genotype. The prevalence of women with surgical menopause was highest in women with the PP genotype, lower in the heterozygous Pp women, and lowest in women with the pp genotype (Table 2). This overrepresentation was most notable for the procedure hysterectomy. As shown in Table 2, the overrepresentation of women with surgical menopause among those with the PP genotype corresponded to a significant 2.4 (95% CI, 1.5–3.8) times higher risk compared to that in women in the reference group with the genotype pp. When we repeated the analysis by type of surgical procedures, we observed the ER genotype-dependent increased risk of surgical menopause to be due to hysterectomy and not oophorectomy. The odds ratios were 1.7 (95% CI, 1.3–2.2) and 0.7 (95% CI, 0.4–1.2)/copy of P allele for hysterectomy and oophorectomy, respectively. Figure 1 shows the lifetime risk of hysterectomy according to the estrogen receptor genotypes as calculated by the Cox proportional hazard function. The PP group had a significantly higher risk of premenopausal hysterectomy, which confirmed the results from the logistic regression analysis.

In Table 3 we stratified the analysis by indication for surgery [validated data not available for 91 of 174 (52.3%) women with surgery]. The predominant indications were menometrorraghia (23%) and uterus myomatosis (fibroids; 43%). Surgical procedures performed for these indications were hysterectomy or hysterectomy plus oophorectomy. There was no oophorectomy solely performed for these indications. We observed that the ER genotype-dependent risk of hysterectomy to be largely due to the higher frequencies

of the indications menometrorraghia and uterus myomatosis (fibroids) in the PP genotype group (Table 3). The odds ratios for the allele dose effect were 2.6 (95% CI, 1.3–5.2) and 1.8 (95% CI 1.1–2.9)/copy of the P allele for menometrorrhagia and uterus myomatosis, respectively.

Discussion

In this population-based study we show for the first time that the common allelic variants of the ER gene are associated with both natural and surgical menopause. Moreover, we provide evidence that women with the PP genotype will undergo hysterectomy due to menorrhagia and fibroids more frequently.

There may have been biases that have lead to incorrect effect estimates. First, the self-report of the age of menopause was determined retrospectively, which has been shown to be unreliable in previous studies (21). Nevertheless, it seems unlikely that this recall bias differs between the ER genotypes. Second, although regional differences in the frequency of hysterectomy have been reported (22–23), it is unlikely that this would have influenced the relation between the ER genotype alleles and the risk of surgical menopause in this
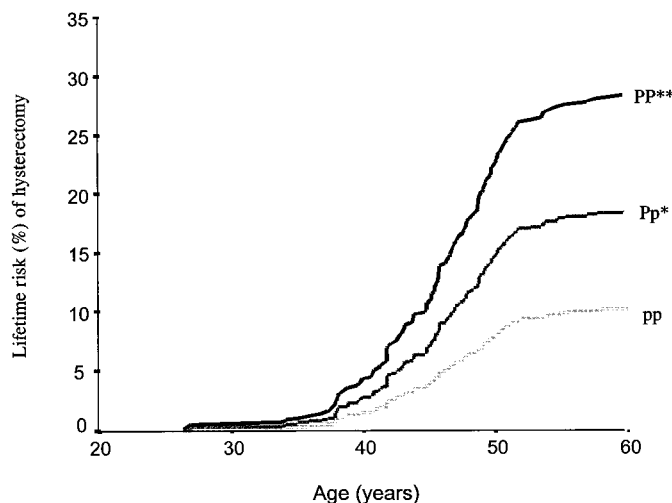

FIG. 1. Lifetime risk (percentage) of premenopausal hysterectomy according to the ER *Pvu*II genotypes. **, $P < 0.001$ (PP genotype *vs.* pp genotype); *, $P = 0.01$ (Pp genotype *vs.* pp genotype; according to the Cox proportional hazard model).

TABLE 2. Frequencies and ODDS ratios (95% CI) for premenopausal gynecological surgical procedures according to ER *Pvu*II genotype

	ER *Pvu*II genotype			Total	
	PP	Pp	pp		
Surgery (%)	55 (26.8)	84 (19.3)	35 (13.5)	174 (19.3)	
Hysterectomy (%)[a]	49 (23.9)	70 (16.1)	24 (9.2)	143 (15.9)	
Ovariectomy (%)	3 (1.5)	12 (2.8)	10 (3.8)	25 (2.8)	
Unknown type (%)	3 (1.5)	2 (0.5)	1 (0.4)	6 (0.7)	
Odds ratio (95% CI)[b]				Allele dose	
Surgery	2.4 (1.5–3.8)	1.5 (1.0–2.4)	1	1.5 (1.2–1.9)	
Adjusted[a]	2.7 (1.6–4.3)	1.7 (1.1–2.7)	1	1.6 (1.3–2.1)	
Hysterectomy	3.1 (1.8–5.2)	1.9 (1.1–3.1)	1	1.7 (1.3–2.2)	
Ovariectomy	0.4 (0.1–1.7)	0.8 (0.3–1.8)	1	0.7 (0.4–1.2)	

[a] Adjusted for age, age at menopause, age at menarche, number of offspring, use of sex steroids, smoking habits, SES, and BMI.
[b] The reference group is women without any premenopausal gynecological surgery.

TABLE 3. Frequency and odds ratio for the indication of surgery according to ER *Pvu*II genotypes

	ER *Pvu*II genotype			Total
	PP	Pp	pp	
Surgical procedures	55	84	35	174
Indication available (%)	29 (52.7)	43 (51.1)	11 (31.4)	83 (47.7)
Subjects used for analysis	179	394	236	809
Menometrorrhagia (%)	9 (5.0)	8 (2.0)	2 (0.8)	19 (2.3)
Uterus myomatosis (%)	12 (6.7)	19 (4.8)	5 (2.1)	36 (4.4)
Other (%)[a]	8 (4.5)	16 (4.1)	4 (1.7)	28 (3.5)
Odds ratio (95% CI)[b]				Allele dose
Menometrorrhagia	6.7 (1.4–31.5)	2.6 (0.5–12.2)	1	2.6 (1.3–5.2)
Uterus myomatosis	3.6 (1.2–10.4)	2.4 (0.9–6.6)	1	1.8 (1.1–2.9)
Other[a]	3.0 (0.9–10.1)	2.6 (0.8–7.8)	1	1.6 (0.9–2.8)

[a] Other includes prolapsed uteri, malignancy, ovarian cyst, or other uncommon diagnosis.
[b] The reference group is women without any premenopausal gynecological surgery.

study. In addition, the allele frequencies of the ER *Pvu*II polymorphism did not differ from frequencies reported previously by others and therefore argue strongly against selection bias in our population (24). Third, although the frequencies of missing data on indication differed among the three genotypes, it is unlikely that the reason for missing these data differed among the ER genotypes. However, in view of the low frequency (48%) of validated data on indication for surgery, confirmation of our observations in another population with more extensive clinical and pathological data is needed to provide more robust risk estimates regarding indication. Finally, the ER genotype-dependent effect on end points of menopause may have biased the results, because women included in the study were probably healthier due to the inclusion and exclusion criteria applied. However, as far as we know, no studies have been published about the association of the ER gene and any of these criteria. Moreover, a separate analysis for the total cohort of the Rotterdam study (n = 4878 women) did not show a relation between the inclusion and exclusion criteria and age at menopause (data not shown).

Evidence that genetic factors are related to age at menopause has been observed previously in family and twin studies (10–12). However, until now, only a limited number of genes have been studied in association with the onset of menopause. One study showed that in a single family four woman had an interstitial deletion of the long arm of X-chromosome, which was associated with premature ovarian failure and premature menopause (25). Furthermore, a link between menopause and the galactose-1-phosphate uridyl transferase gene was reported (26). This study, however, had limited generalizability due to population admixture and the small number of subjects examined.

In the present study we observed that common variations of the ER gene are associated with disorders of the uterus. This supports the hypothesis that a common factor is involved in the pathogenesis of abnormal bleeding and uterine fibroids (27). The present observation seems logical due to the fact that the ER has been identified in endometrium, myometrium, and fibroids (15). In addition, estrogens have a direct effect on the uterus, which is emphasized by the fact that ER knockout mice demonstrated lack of uterine response to estrogen treatment compared to wild-type animals (28).

Although it is unclear how the common allelic variations of the ER gene influence the action of the ER for its ligand estradiol, the present study showed an earlier onset of natural menopause and a higher prevalence of hysterectomy due to fibroids and menorrhagia in women carrying the P allele. This suggests a higher responsiveness of this ER gene allele to estrogen, which might affect the biological response and ultimately leads to irregularities in the differentiation and proliferation of endo- and myometrial cells. However, it must be emphasized that it is presently unclear what is the molecular mechanism of the association we here report. Because the *Pvu*II RFLP is an anonymous polymorphism, it seems likely that the P allele is in linkage disequilibrium with a truly causative sequence variation elsewhere in the ER gene or even in another nearby gene. To elucidate the precise molecular mechanism, extensive sequence analysis and functional studies of the ER gene variants are needed.

In conclusion, we have obtained evidence that a common allelic variation in the ER gene is associated with age at menopause as well as hysterectomy. This raises the possibility that genotyping at the polymorphic *Pvu*II ER gene may provide information on susceptibility to uterine disorders leading to early onset of menopause, which eventually might lead to other clinical entities, such as osteoporosis, cardiovascular diseases, and ovarian cancer.

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