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Factor V Leiden mutation accelerates the onset of natural menopause

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ABSTRACT

Objective: Smoking is consistently associated with a younger age for menopause. Although this may be because of the direct toxic effects of tobacco smoke on follicles, we hypothesize that there may also be a relationship between smoking and a vascular origin of early menopausal onset. Several lifestyle factors have been investigated, but never factors of the clotting cascade. The objective of this study, then, was to determine the effect of factor V Leiden mutation and smoking with respect to age at menopause.

Design: Data were used from a subset of 373 postmenopausal participants of a Dutch population-based cohort, born between 1911 and 1925. All women had experienced natural menopause, without use of hormone replacement therapy.

Results: Female carriers of the factor V Leiden mutation (n = 14) reported the onset of menopause at an earlier age than noncarriers (n = 359; difference, 3.1 years; 95% CI: 0.3, 5.9). Smoker carriers (n = 5) were 4.3 years younger at menopause than smoker noncarriers (n = 92; 95% CI: 0.9,7.6). In nonsmokers, this relationship was less strong.

Conclusions: We found that the factor V Leiden mutation was related, but not statistically significant, to an earlier age at menopause; smoking possibly enhances this effect. The mutation can be one of the genetic determinants of menopausal age operating through a vascular mechanism.

Keywords: Age at menopause – Determinants – Factor V Leiden – Smoking.

The onset of natural menopause varies between 40 to 60 years, with a median age of 50 to 51.5 years,[1,2] and may be caused by the exhaustion of the oocyte/follicular reserve.[3] Early menopause has been associated with several chronic diseases later in life. An annual risk reduction of 2% (95% CI: 1%, 3%) each year that menopause is delayed has been reported for cardiovascular mortality.[4] Also, osteoporosis is associated with an earlier age at menopause;[5] Japanese women who experience menopause after age 50 have a decreased risk of low bone density (odds ratio 0.70; 95% CI: 0.50, 0.99).[6] On the other hand, an early end of reproductive life might lower the risk of breast cancer; for each year increase in age at menopause, the relative risk of breast cancer is 1.03 (95% CI: 1.02, 1.03).[7]

Several determinants of menopausal age, including various lifestyle factors, have been investigated. However, these factors only explain less than 3% of the large variation in age at menopause.[2] Genetic factors could well be responsible for the remaining variation in age at menopause, as twin and sibling studies have shown heritabilities varying between 50% and 85%.[8-10]

The most important lifestyle factor affecting age at menopause discovered so far is smoking. Women who

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smoke reach menopause approximately 0.8 to 2 years earlier than nonsmokers.[11-13] Enhanced follicular depletion from a toxic effect may explain some of this effect of smoking. Recent observations on the effect of the biologically active ingredients of cigarette smoke in mice demonstrate an up-regulation of the pro-apoptosis genes in ovaries, which results in an increased depletion of the ovarian reserve.[14]

Smoking is a well-established risk factor for vascular disease, which led us to hypothesize that a vascular pathway might also be involved in ovarian aging. Smoking has been identified as one of the most important determinants of fibrinogen levels,[15] and fibrinogen levels in turn have been associated with occlusive thrombotic disease.[16] Although findings are still speculative in this field, it may be interesting to test whether thrombosis also affects highly vascularized organs like the ovaries. Factor V Leiden mutation is known for its association with thrombotic events.[17] Heterozygosity for the factor V Leiden mutation is found in 2% to 4% of the people in Western Europe, including the Dutch population. The purpose of this study was to examine the individual and combined effects of the factor V Leiden mutation and smoking on age at natural menopause.

METHODS

Population

Women were recruited from participants of an experimental breast cancer screening program (DOM, Diagnostic Investigation Mamma cancer), carried out between December 1974 an October 1980. Participants were born between 1911 and 1925 and resided in the city of Utrecht, The Netherlands. Women were invited for repeated examinations at 1-, 2-, and 4-year intervals. The source population consisted of 12,239 women who attended at least the first and second screening because, at the second exam, a questionnaire on lifestyle factors, including smoking, was administered. During the first visit, urine samples were collected and stored at −20°C.

A random sample of 650 women was selected to determine the factor V Leiden polymorphism. All women gave informed consent to use their data and urine for future scientific research. The Institutional Review Board of the University Medical Center Utrecht, The Netherlands, approved the study to determine factor V Leiden.

Genotyping

DNA was isolated from 50 mL urine.[18] Factor V Arg/Gln506 genotype was determined from each DNA fraction using Polymerase Chain Reaction and hybridization with antigen specific oligonucleotides. The antigen specific oligonucleotide for factor V Arg506 was '3-TGGACAGGAAGGAATAC-5' and for Factor V Gln506 '3-GGACAGGCGAGGAATAC-5'. Dots were visualized on x-ray films (DuPont, Brussels, Belgium) after overnight radiation. For 47 women, no urine was stored; and in 41 women, either DNA isolation or genotyping for factor V Leiden was not successful.

Determinants

Smoking status was assessed at the second screening visit, and women were defined as smokers if they reported being current smokers at that time. Menopausal status was self-reported and questioned at the first and fifth screening visit. The first mention of menopausal age was included in the analysis. Menopause was defined as at least 12 consecutive months of amenorrhea. Women were excluded if they were still premenopausal at the fifth examination (30 women) or if they had undergone either a hysterectomy or bilateral ovariectomy (103 women). Women who did not menstruate after cessation of oral contraception or who reported having used hormone replacement therapy (HRT) were also excluded (47 women), and 8 women were excluded because data on menopausal status were missing. The resulting study population consisted of 374 women with a natural menopause.

Statistical analysis

Descriptive analysis was used to describe the study population. Mean age at menopause was computed for factor V Leiden carriers and noncarriers. A *t* test with nonequal variances was used to compare mean menopausal ages of carriers and noncarriers. Stratification according to smoking was performed to determine the presence of effect modification. Interaction was tested using a linear regression model, with menopausal age as a dependent variable and smoking, factor V Leiden, and the multiplicative factor of smoking and carriership included as independent variables.

All statistical analyses were performed using SPSS 10.1 for Windows.

RESULTS

The allele frequency of the factor V Arg506Gln polymorphism was 4%. One homozygote was found

TABLE 1. *Menopausal age according to smoking status and factor V Leiden mutation in 373 women*

	Number	Mean age (SE)	95% CI[a]	*P*-value[a]
Nonsmokers	276	50.3 (0.22)	−0.6, 1.1	0.55
Smokers	97	50.1 (0.38)		
Noncarriers' factor V Leiden	359	50.4 (0.19)	0.3, 5.9	0.035
Carriers' factor V Leiden	14	47.3 (1.29)		
Nonsmoking				
Noncarriers' factor V Leiden	267	50.4 (0.22)	0.0, 4.8	0.05
Carriers' factor V Leiden	9	48.0 (1.69)		
Smokers				
Noncarriers' factor V Leiden	92	50.3 (0.38)	0.9, 7.6	0.013
Carriers' factor V Leiden	5	46.0 (2.07)		

CI, confidence interval; SE, standard error.
[a]Two-sided *t*-test.

but was left out of the analyses because of the small number. The mean age of the remaining 373 women at enrollment was 58.0 (SD 4.1) years; it was equal for carriers and noncarriers.

All women with the factor V Leiden mutation reported a postmenopausal status at the first screening visit. For noncarriers, 21 women were premenopausal at the first exam but reported to be postmenopausal at the fifth examination.

The mean age at menopause for the whole group was 50.2 years (SD 3.7, range 39-60). The smoking percentage was 35.7% in carriers and 25.7% in noncarriers ($P = 0.4$). Results are shown in Table 1.

Smoking was associated with a slightly decreased age at menopause, although this was not statistically significant. Women carrying the mutation had menopause earlier than noncarriers (mean age at menopause of 47.3 years v 50.4 years; 95% CI for the difference in means: 0.3, 5.9). Stratified for smoking, we observed that this relationship was just significant in nonsmokers (difference 2.4 years, 95% CI: 0.0, 4.8) but was strong and statistically significant in smoking women (difference 4.3 years, 95% CI: 0.9, 7.6). Modification by smoking of the association between factor V Leiden carriership and menopausal age was also tested in a linear regression model but was nonsignificant ($P = 0.4$).

DISCUSSION

This study suggests that the factor V Leiden mutation was associated with an earlier age at menopause, and that smoking may possibly enhance this effect. Despite the small number of individuals investigated, the phenotypic effect of a reduction in age at natural menopause of 3 years in carriers of factor V Leiden was substantial. The finding suggested modification by smoking, as the effect was strong in smokers and borderline significant in nonsmokers. However, the interaction term in the regression model was not significant, which may have been due to the small numbers in the strata.

One limitation of our study is that smoking behavior was reported at study recruitment, when most women were already postmenopausal. However, from demographic studies it is known that the quitting rate of women 35 to 50 years of age in the '70s was about 1% to 3%, depending on educational level.[19] This small percentage suggests that smoking behavior at study recruitment probably still reflects the smoking status at menopausal transition. Although some misclassification of smoking behavior during menopausal transition may have occurred, this is most likely nondifferential, ie, women with factor V Leiden did not stop smoking more often than noncarriers or the reverse. Nondifferential misclassification predominantly leads to underestimation of the true association.

The association between smoking and menopausal age has earlier been studied in the DOM population, and a significant relationship was found; smoking decreased menopausal age by 1.2 years ($P < 0.001$).[20] Our sample was apparently too small to replicate this finding.

Because menopausal status is almost always measured retrospectively and by questionnaires, this inherently can give problems with validation and reproducibility. However, these aspects have been reported previously in this cohort.[21] The reproducibility was studied in 4,892 women who attended the first and the fifth screening, with an interval of 7.5 years. For 80% of the sample, the two reports differed by at most 1 year. The findings were similar to those of the Nurses' Health Study.[22] Furthermore, the number of years since menopause decreases the validity of reporting menopausal age; the percentage of women reporting their true age at menopause was higher when less

time had passed since menopause. Both under-as overestimations occurred when more time had passed since menopause, but the mean of the self-reported menopausal age was higher than the true menopausal age.[21] The average time since menopause in our study was 7 years for noncarriers and 10 years for carriers. This suggests that carriers may be less accurate in self-reported menopausal age than noncarriers, but it also suggests that our estimate could even be an underestimation of the real association because the true menopausal age of the carriers may have occurred earlier than they reported.

In general, blood samples are used to isolate DNA. However, when blood is unavailable, frozen urine samples can be considered as a useful and valid method of obtaining DNA in large cohort studies, as published recently.[18]

There is currently no biological explanation for why factor V Leiden would lead to an early menopause, but we hypothesize a possible vascular pathway. The factor V Leiden mutation is responsible for a reduced factor Va inactivation, leading to a reduced inhibition of prothrombin. It is known that this mutation leads to an increased risk of venous thrombosis.[17] Furthermore, in two pooled analyses, factor V Leiden mutation was also significantly associated with the risk of myocardial infarction.[23,24]

Moreover, the risk of premature ischemic stroke has been reported to be higher for female carriers of the factor V Leiden mutation than for male carriers.[25] This suggests the possibility of an interaction of this genotype with female sex hormones. Estrogens are known to further lower the inactivation rate of factor Va, which could explain the interaction with sex.[26]

Estrogen levels in the follicular fluid can cyclically reach high levels. If estrogen and factor V interact, then the effect must be most pronounced locally in and near the ovaries. Ovaries are highly vascularized organs, and angiogenesis of developing follicles is known to play an important role in both the follicular and luteal phase.[27] Enhanced activation of factor V, as exhibited by the factor V Leiden mutation and by high estrogen levels,[26] may lead to formation of ovarian microthrombi and subsequently affect the vascularization of the follicular system and reduce oxygen levels of the microenvironment of the follicles. Infarction of developing follicles could lead to accelerated depletion of the oocyte/follicle pool that would be translated into an earlier onset of menopause.

Miscarriages have been reported to occur more frequently in female carriers of the factor V Leiden mutation.[28] This also may be explained by adverse effects of the microenvironment of follicles because oocytes from severely hypoxic follicles have been associated with abnormalities in chromosomes.[27]

The relationship between thrombosis and smoking is still controversial;[29] nevertheless, fibrinogen levels are raised in smokers, possibly increasing the susceptibility of smokers to thrombosis.[30] Moreover, in premenopausal women, the combination of smoking and carriership of the factor V Leiden mutation leads to a much higher risk of myocardial infarction compared with smoking or carriership of factor V Leiden mutation alone.[31]

Early menopause has been associated with cardiovascular disease, but some risk factors of cardiovascular disease, like smoking or factor V Leiden mutation, may affect menopausal age. If this effect is proven in future studies, underlying cardiovascular disease might predispose a woman to early menopause.

Another less likely explanation would be that factor V Leiden may be in linkage disequilibrium with another gene involved in ovarian aging. This then implies that this gene must be located very near to the factor V Leiden mutation on chromosome 1.

If subsequent studies confirm our results, factor V Leiden would be the second genetic factor shown to contribute to variation in age at natural menopause. The first was the estrogen receptor 1 (ESR1) polymorphism, in which the minor allele frequency is 47% and responsible for a 1-year decrease in age at natural menopause in homozygotes.[32] The factor V Leiden mutation has a much smaller mutant allele frequency, but its relative impact on menopausal age is much larger. Future research should also include ESR1 polymorphism to test interaction between estrogen sensitivity and a disabled clotting system.

CONCLUSION

The factor V Leiden mutation may be associated with an earlier age at menopause. The mutation can be one of the genetic determinants of menopausal age. Our finding suggests new lines of investigation of the possible role of clotting factor polymorphisms in determining age at natural menopause. More studies are needed to accept this prospect with confidence.

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