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Prothrombotic mutations, family history and the risk of thrombosis in postmenopausal women: implications for hormone replacement therapy

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Key words: HORMONE REPLACEMENT THERAPY, FAMILY HISTORY, PROTHROMBOTIC MUTATION, FACTOR V LEIDEN, PROTHROMBIN G20210A VARIANT, METHYLENETETRAHYDROFOLATE REDUCTASE C677T VARIANT

ABSTRACT

Objective Hormone replacement therapy (HRT) is acknowledged as the gold standard for the alleviation of climacteric vasomotor symptoms. Prothrombotic genetic variants have been suggested to increase thrombotic risk among HRT users. The aim of the study was to determine whether a positive family history may identify a genetic predisposition for thrombosis in women before prescribing HRT.

Methods From January 2005 to May 2009, we consecutively enrolled 145 asymptomatic women (mean age 51.2 ± 5.4 years) without previous episodes of venous and/or arterial thrombosis referred to our Genetics Research Unit before starting HRT. A detailed family history was reconstructed and we identified 48 women (33.1%) with a positive family history, defined as venous thromboembolism and/or stroke or heart attack, in first-degree relatives before 60 years for men and 65 years for women. A group of 121 women (mean age 54.0 ± 9.1 years) with an episode of venous and/or arterial thrombosis was also included. Genetic screening for factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase C677T polymorphisms was performed.

Results The frequency of factor V Leiden or prothrombin G20210A mutations was significantly higher both in asymptomatic women with a positive family history (16.7% vs. 2.1%, $p = 0.001$) and in patients with thrombosis (12.4% vs. 2.1%; $p = 0.005$) compared with asymptomatic women without a family history. Multivariate regression analysis showed a synergic effect between the presence of one prothrombotic mutation and family history on the risk of thrombosis (odds ratio 3.7, 95% confidence interval 1.9–7.2).

Conclusions A positive family history of thrombosis is a sensitive indicator for selected genetic testing in high-risk women before starting HRT.

INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality for both men and women. It is well-known that the risk of cardiovascular disease in women rises sharply after menopause[1,2]. This increased risk is mainly attributed to the reduction in levels of the female sex hormone estrogens, suggesting that they have cardioprotective effects[3–5]. Nevertheless, the initial findings of the randomized studies (Heart and Estrogen/progestin Replacement Study (HERS) and Women's Health Initiative (WHI)) generated concern about the detrimental effect of hormone replacement therapy (HRT)

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on cardiovascular risk[6,7]. However, re-analysis of the WHI data has shown that there is a likely beneficial effect on the cardiovascular system for women who begin treatment with HRT at or near the time of the menopause[8,9]. In fact, according to the recent consensus statement of European cardiologists and gynecologists, hormone therapy is inappropriate for older postmenopausal women no longer displaying menopausal symptoms[10]. Congruent trends suggested additional benefit, including reduction of overall mortality and coronary artery disease, in women starting HRT between the ages of 50 and 59 years or less than 10 years after the onset of menopause[11]. Anyway, assessments of benefit and risk in younger perimenopausal women is difficult because it is based on lower levels of evidence[10,11].

Recently, it has also been suggested that the risk/benefit ratio may depend on the genetic predisposition of women, in particular the presence of inherited thrombotic risk factors such as factor V Leiden and G20210A prothrombin (PT G20210A) gene mutation[12]. In fact, several studies reported a 13–16-fold increased risk of venous thromboembolism during HRT among women with factor V Leiden[13–16]. Furthermore, women on HRT with factor V Leiden or PT G20210A mutation have a remarkably increased risk of myocardial infarction or stroke[17–19]. In addition, a common C677T polymorphism in the *MTHFR* gene has been reported to be a risk factor for deep venous thrombosis[14,20]. Interestingly, women with MTHFR 677T variants do not show a decrease in homocysteine plasma levels in response to HRT when compared to women with the 677CC genotype, suggesting a pharmacogenetic variability on the cardiovascular effects[21]. At the moment, there is no clinical indication to screen women for genetic risk factors before prescribing hormone therapy. The predictive value of a positive family history of early thrombotic events may be useful in identifying high-risk women, but the effectiveness of this approach has not yet been proved.

The objective of this study was to evaluate the sensitivity of a positive family history of thrombosis in order to identify the presence of prothrombotic mutations in asymptomatic women before prescribing HRT.

METHODS

Study population

From January 2005 to May 2009, we consecutively selected 145 asymptomatic women (mean age 51.2 ± 5.4 years) without previous episodes of venous and/or arterial thrombosis who were consecutively referred to our Genetics Research Unit by gynecologists before starting HRT. In addition, we included a group of 121 women (mean age 54.0 ± 9.1 years) who were admitted to our Clinical Departments with an episode of venous and/or arterial thrombosis. Deep vein thrombosis was objectively diagnosed on the basis of the results of at least one of these tests: venography, compression ultrasonography, color Doppler, or plethysmography. A diagnosis of pulmonary embolism was made on the basis of the results of ventilation-perfusion lung scan, pulmonary angiography, computerized tomography scan or magnetic resonance imaging. Myocardial infarction was diagnosed by qualified cardiologists on the basis of typical electrocardiography changes, elevated cardiac markers and clinical history. Ischemic stroke was documented clinically by a neurologist and radiographically by either cranial computed tomography or magnetic resonance imaging. Venous thromboembolism and arterial thrombosis were diagnosed in 20 women (16 suffered from deep vein thrombosis and four from pulmonary embolism) and 101 women (85 with acute myocardial syndrome and 16 with ischemic stroke), respectively. No patients had been taking oral contraceptives or HRT at the time of event. At the moment of blood sampling, a detailed family history was reconstructed for each woman. A positive family history was defined as venous thromboembolism and/or stroke or heart attack in first-degree relatives before 60 years for men and 65 years for women. Women were also asked about additional acquired thrombotic risk factors such as hypertension, dyslipidemia, diabetes mellitus and cigarette smoking. The following definitions were utilized: hypertension, blood pressure $\geq$140/90 mmHg (confirmed by measurements on several occasions) or antihypertensive therapy; dyslipidemia, low density lipoprotein cholesterol $\geq$130 mg/dl, high density lipoprotein cholesterol $<$35 mg/dl, triglycerides $\geq$200 mg/dl or use of lipid-lowering medications. The diagnosis of diabetes mellitus was established according to World Health Organization criteria: fasting plasma glucose $\geq$7.0 mmol/l or antidiabetic medication. Smokers were classified as individuals who smoked at least three cigarettes per day at the time of analysis, past smokers had quit smoking for at least 6 months, and no-smokers were individuals who had never smoked. Accordingly, each risk factor was coded as either present or absent. Written informed consent was obtained from all study participants.

Genotyping

Screening for inherited prothrombotic conditions, including the G1691A mutation in the factor V Leiden gene, the G20210A mutation within the 3′-untranslated region of the PT gene, and the C677T MTHFR polymorphism, were performed in all subjects by using a multiplex allele-specific PCR assay (Nuclear Laser Medicine, srl), as previously described[12,22].

Statistical analysis

All statistical analyses were conducted using the Statview statistical package, version 5.0.1 (SAS Institute, Abacus Concepts, Inc., Berkeley, CA, USA). Data were expressed as the mean $\pm$ standard deviation. Differences between the means of the two continuous variables were evaluated by Student's *t*-test. Differences in non-continuous variables and genotype distribution were tested by χ^2 analysis. Uncondi-

tional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). A p value of 0.05 was considered statistically significant.

RESULTS

Thrombosis-free cohort

Anamnestic data demonstrated a positive first-degree family history of venous and/or arterial thrombotic events in 48 women (33.1%). No significant differences were observed for traditional thrombotic risk factors between women with or without family history (Table 1). The prevalence of prothrombotic mutations, factor V Leiden and PT G20210A, was higher in thrombosis-free women with a positive family history compared with women without (10.4% vs. 1.0%; $\chi^2 = 7.1$, $p = 0.008$ for factor V Leiden, and 6.2% vs. 1.0%; $\chi^2 = 3.3$, $p = 0.07$ for PT G20210A, respectively). The frequency of women carrying at least one prothrombotic mutation (factor V Leiden or PT G20210A) was significantly higher in the group with a positive family history than in the group without (16.7% vs. 2.1%, $\chi^2 = 10.7$, $p = 0.001$). No significant differences were observed for the MTHFR 677TT genotype (29.2% vs. 25.8%; $p = 0.7$) (Table 1).

Cohort of women with personal history of thrombosis

As expected, the women who had experienced previous thrombotic events showed a higher prevalence of hypertension ($p < 0.0001$), dyslipidemia ($p < 0.0001$), diabetes ($p = 0.0001$), and family history ($p < 0.0001$) compared with the thrombosis-free group (Table 2).

Table 1 Clinical and demographic characteristics of the thrombosis-free cohort of women. Data are given as mean ± standard deviation or n (%)

	Positive family history ($n = 48$)	*Negative family history* ($n = 97$)	*p Value*
Age (years)	52.0 ± 4.9	50.7 ± 5.6	0.2
Smoking habit			0.7
no smoking	31 (64.6)	65 (67.0)	
past-smoking	7 (14.6)	10 (10.3)	
smoking	10 (20.8)	22 (22.7)	
Hypertension	5 (10.4)	18 (18.6)	0.2
Dyslipidemia	11 (22.9)	24 (24.7)	0.8
Diabetes mellitus	0 (0)	2 (2.1)	0.3
Body mass index (kg/m^2)	24.7 ± 4.3	26.0 ± 5.3	0.2
Homocysteine (μmol/l)	11.1 ± 7.2	11.0 ± 6.0	0.9
677TT MTHFR genotype	14 (29.2)	25 (25.8)	0.7
Factor V Leiden variant	5 (10.4)	1 (1.0)	0.008
Prothrombin 20210A variant	3 (6.2)	1 (1.0)	0.07

The frequency of either factor V Leiden or PT G20210A prothrombotic mutation was also higher, although not significant, in these patients when compared with the thrombosis-free group (12.4% vs. 6.9%). No significant alterations were observed with regard to other coagulation defects, including protein C, protein S and antithrombin III.

Patients with a positive family history also had a higher prevalence of prothrombotic mutations compared with those without (15.7% vs. 7.8%, $p = 0.2$).

Moreover, we observed that the frequency of either factor V Leiden or PT G20210A variant was significant higher in this group of patients when compared to thrombosis-free women without a family history (12.4% vs. 2.1%; $\chi^2 = 7.9$, $p = 0.005$). On the contrary, no significant difference in the proportion of prothrombotic defects was observed between patients and thrombosis-free women with a positive family history (12.4% vs. 16.7%; $\chi^2 = 0.5$, $p = 0.5$) (Figure 1). The overall odds ratio for thrombotic events in the presence of one prothrombotic mutation was 2.0 (95% CI 0.9–4.4, $p = 0.09$). Multivariate regression analysis showed a synergic effect between the presence of one prothrombotic mutation and family history on the risk of thrombosis (OR 3.7, 95% CI 1.9–7.2) (Table 3).

DISCUSSION

Our data showed a high sensitivity of a positive family history of early thrombosis in identifying prothrombotic mutations in asymptomatic women before starting HRT. In the last decades, the potential cardioprotective effects of HRT have been extensively investigated, with controversial results[23]. Observational studies have suggested a significant reduction in cardiovascular events after HRT, but large clinical trials have

Table 2 Demographic and genetic characteristics of the study population according to the presence of thrombotic events. Data are given as mean ± standard deviation or n (%)

	Thrombosis-free women ($n = 145$)	*Patients* ($n = 121$)	*p Value*
Age (years)	51.2 ± 5.4	54.0 ± 9.1	0.002
Smoking habit			0.5
no smoking	96 (66.2)	80 (66.1)	
past-smoking	17 (11.7)	19 (15.7)	
smoking	32 (22.1)	22 (18.2)	
Hypertension	23 (15.9)	51 (42.1)	<0.0001
Dyslipidemia	35 (24.1)	68 (56.2)	<0.0001
Diabetes mellitus	2 (1.4)	16 (13.2)	0.0001
Family history	48 (33.1)	73 (60.3)	<0.0001
Body mass index (kg/m^2)	25.6 ± 5.0	26.1 ± 5.5	0.5
677TT MTHFR genotype	39 (26.9)	26 (21.5)	0.3
Factor V Leiden variant	6 (4.1)	6 (5.0)	0.7
Prothrombin 20210A variant	4 (2.8)	9 (7.4)	0.08

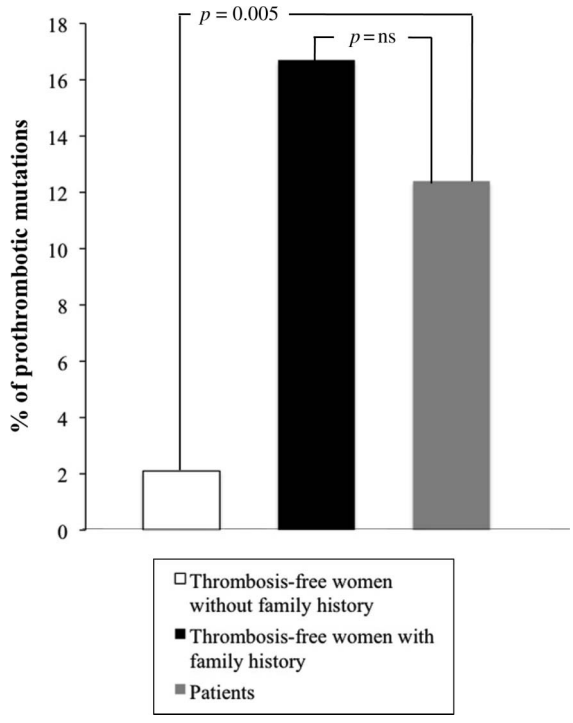

Figure 1 Percentage of prothrombotic mutations according to family and personal history of thrombosis

Table 3 Risk factors for thrombotic events according to multiple logistic regression analysis in the study population

Risk factors	*Odds ratio (95% confidence interval)*	*p value*
Age (years)	1.0 (0.9–1.1)	0.7
Smoking habit	1.0 (0.6–1.6)	0.9
Hypertension	3.8 (1.5–9.7)	0.005
Dyslipidemia	4.4 (1.9–10.0)	0.0003
Diabetes mellitus	6.5 (0.8–54.8)	0.08
Body mass index	0.9 (0.9–1.0)	0.4
Family history	3.1 (1.9–5.1)	0.0001
Prothrombotic mutation	4.2 (0.9–19.3)	0.05
Family history + prothrombotic mutation	3.7 (1.9–7.2)	0.0001

demonstrated that hormone therapy may increase cardiovascular risk in late postmenopausal women[3,7,24–29]. The apparent discrepancy between these results recently seems to be explained in terms of time to initiation of therapy[8–11,30]. Estrogen therapy used from the time of menopause onward may be cardioprotective because of the responsiveness of the endothelium to estrogen that buffers the detrimental effects on coagulation. However, if HRT is instituted after endothelial damage has occurred in late postmenopausal women, the beneficial effects on the vessel wall are not observed because of the predominance of the pro-coagulant or plaque-destabilizing effects, leading to an increase in cardiovascular risk[31,32]. In fact, in a recent statement by European cardiologists and gynecologists[10], Practice point 6 states that 'Cardiovascular risk associated with hormone therapy exceeds the benefit in elderly post-menopausal women. In treating the younger, perimenopausal woman for menopausal symptoms, the benefits should be weighed against the potential risks of hormone replacement therapy.' In just this subset of women, other concomitant factors may be involved, including the presence of cardiovascular risk factors or individual susceptibility. Recently, it has been suggested that the benefit of HRT could be obscured by an increased risk of thrombotic events in women genetically predisposed to thrombotic complications[12]. In particular, the presence of the factor V Leiden and/or prothrombin gene mutation may increase the risk for thrombosis in HRT users[12,14–19]. The Estrogen and Thromboembolism Risk (ESTHER) study confirmed the associations between increased venous thromboembolism risk in postmenopausal women and the presence of either factor V Leiden or PT G20210A mutation[13]. Moreover, among postmenopausal hypertensive women, the association between HRT use and myocardial infarction risk is higher in the presence of the prothrombin G20210A variant[18]. In such women, the safety of hormone therapy is questionable and a genetic screening for thrombotic risk factors might permit a better assessment of the risks and benefits associated with HRT[12]. In the last decade, genetic testing for the identification of individual susceptibility has become common practice[20]. However, there is considerable debate over the psychological and social problems of genetic testing in asymptomatic individuals[20]. The usefulness of genetic screening in women prior to prescribing hormone therapy is not yet well defined. As a consequence, indiscriminate thrombotic screening is deemed not to be cost-effective. Our findings strongly support the belief that a selective screening in women with a personal and/or family history of venous and arterial thrombosis is strongly recommended, in an attempt to increase cost-effectiveness and avoid denial of the benefits of hormonal therapy to many women. In the literature, there is no agreement on the value of the family history for the selection of patients who should be screened for thrombophilia. Some reports have demonstrated a low sensitivity and positive predictive value of family history of venous thromboembolism for identifying women with thrombophilia, suggesting that a policy of selective screening may therefore miss a substantial number of women at increased risk of thrombotic complications when taking oral contraceptives[33,34]. However, a recent meta-analysis showed that selective screening based on previous personal and/or family history of venous thromboembolism was more cost-effective in all four different patient groups considered: women prior to prescribing oral contraceptives or HRT, women at the onset of pregnancy, and patients prior to major orthopedic surgery, compared with no screening[35].

An undoubted limitation of our study is the low number of women, which may make the results of our interaction analysis statistically unstable, although the present study is sufficiently powered in the case of odds ratios > 3.

In conclusion, hormone therapy should be discussed with each patient, taking into account the prevalence and the relevance of the patient's symptoms and risk factors. Each woman should be counselled regarding the risks and perceived benefits of the therapy. Genetic screening provides a useful tool for gynecologists in order to improve the safety and efficacy of individualized HRT and might be advisable in women with a family history of thrombotic events in order to minimize adverse drug reactions in high-risk subjects.

Conflict of interests The authors declare no conflict of interest.

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