Prothrombotic Mutations, Hormone Therapy, and Venous Thromboembolism Among Postmenopausal Women

Impact of the Route of Estrogen Administration

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Background—Oral estrogen increases the risk of venous thromboembolism (VTE) in postmenopausal women, particularly in those with a prothrombotic mutation. Transdermal estrogen may be safe with respect to VTE. We investigated the impact of the route of estrogen administration on the association between a prothrombotic mutation (factor V Leiden or prothrombin G20210A mutation) and VTE risk.

Methods and Results—We performed a multicenter case-control study of VTE among postmenopausal women who were enrolled in 1999 through 2004 at 7 clinical centers in France. We recruited 235 consecutive patients with a first documented episode of idiopathic VTE and 554 controls. Factor V Leiden was associated with a 3.4-fold–increased risk of VTE (95% confidence interval [CI], 2.0 to 5.8), and a prothrombin mutation was associated with a 4.8-fold–increased risk of VTE (95% CI, 2.5 to 9.4). Oral but not transdermal estrogen was associated with an increased risk of VTE (odds ratio [OR], 4.3; 95% CI, 2.6 to 7.2; and OR, 1.2; 95% CI, 0.8 to 1.7, respectively). After adjustment for potential confounding factors, the combination of either factor V Leiden or prothrombin G20210A mutation and oral estrogen gave a 25-fold–increased risk of VTE compared with nonusers without mutation (95% CI, 6.9 to 95.0). However, the risk for women with prothrombotic mutation using transdermal estrogen was similar to that of women with a mutation who were not using estrogen (OR, 4.4; 95% CI, 2.0 to 9.9; and OR, 4.1; 95% CI, 2.3 to 7.4, respectively).

Conclusions—In contrast to oral estrogen, transdermal estrogen does not confer additional risk on women who carry a prothrombotic mutation. The safety of transdermal estrogen has to be confirmed in randomized trials. **(*Circulation*. 2005;112:3495-3500.)**

Key Words: embolism ■ epidemiology ■ genetics ■ hormones ■ thrombosis

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Observational data and randomized trials have consistently shown that oral estrogen with or without progestogen was associated with an increased risk of venous thromboembolism (VTE).[1] However, the Estrogen and Thromboembolism Risk (ESTHER) study has recently suggested that transdermal estrogen had no effect on VTE risk among postmenopausal women.[2] Factor V Leiden and prothrombin G20210A mutation are the 2 most common genetic defects associated with an increased risk for VTE.[3,4] These prothrombotic mutations may have an impact on the relationship between VTE and hormone therapy. Two case-control studies have shown that the combination of factor V Leiden and oral estrogen increased the risk of VTE ≈15-fold.[5,6] Recently, the effect of hormone therapy on the VTE risk among postmenopausal women has been studied in the Women's Health Initiative (WHI) Estrogen Plus Progestin clinical trial.[7] In this randomized trial, women who had factor V Leiden and used estrogen plus progestin had a 6.7-fold–increased risk of VTE (95% confidence interval [CI], 3.1 to 14.5) compared with women in the placebo group without the

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TABLE 1. Characteristics of Patients With VTE and Controls

	Patients (n=235)	Controls (n=554)	*P*
Age, y	61.8 (6.8)	61.3 (6.6)	0.4
BMI, kg/m^2*	26.7 (5.4)	24.6 (4.7)	<0.0001
BMI >30 kg/m^2, n (%)*	49 (20.9)	69 (12.5)	0.0001
Current smokers, n (%)*	25 (10.7)	70 (12.7)	0.4
Educational level beyond secondary, n (%)†	36 (15.9)	113 (20.4)	0.1
Personal history of varicose veins, n (%)	129 (54.9)	252 (45.5)	0.02
At least 1 risk factor, n (%)‡	72 (30.8)	141 (25.5)	0.12

Values are mean (SD) when appropriate.
*Data for 1 patient are missing.
†Data for 9 patient and 1 control are missing.
‡Hypertension, obesity, or diabetes.

mutation. However, these results apply to combined oral estrogen with medroxyprogesterone acetate and are not necessary relevant to other hormone regimens. Furthermore, the transdermal route of estrogen administration was not investigated. Therefore, we have evaluated the impact of the route of estrogen administration on the association between prothrombotic mutations and VTE among the participants in the ESTHER case-control study.[2] This article is based on cases and controls recruited through July 2004.

Methods

Study Population

A detailed description of the ESTHER study is given elsewhere.[2] Briefly, it is a multicenter case-control study of VTE among postmenopausal women 45 to 70 years of age who were recruited to examine the impact of the route of estrogen administration (oral or transdermal) on VTE risk.

From 1999 to 2004, 179 hospital cases with a first documented episode of idiopathic VTE (110 with pulmonary embolism [PE] and 69 with deep venous thrombosis [DVT]) were recruited consecutively, and 362 hospital controls were matched by center, 2-year age band, and date of admission. Hospital controls had to have been admitted with an a priori diagnosis unrelated to hormone therapy, including diseases of the eye, ear, skin, respiratory and alimentary tracts, bone and joints, kidneys, infectious diseases, and diabetes. Moreover, between 1999 and 2002, we included 56 outpatient cases (19 PE, 37 DVT) referred to 3 hematology outpatient clinics and their community controls (n=192) selected at random from electoral rolls according to the same criteria and matched by age and area of residence. About 10% of community controls refused to participate in this study. Hospital and outpatient cases with high blood pressure, obesity, or diabetes were also matched to controls with one of these vascular risk factor.

Both hospital and outpatient cases were excluded if they reported a previous episode of VTE, had a contraindication for hormone therapy, or had a predisposing factor for VTE (history within the previous month of surgical intervention, trauma with immobilization for >8 days, illness necessitating bed rest for >8 days, known cancer, systematic inflammatory disease). Outpatient cases were also excluded if they were referred to clinical centers for estrogen advise or known prothrombotic mutations. Controls were subject to the same exclusion criteria as were patients.

Data Collection

Patients and controls were identified without knowledge of estrogen use. Diagnoses of VTE required confirmation by imaging procedures.[2] Events were adjudicated within each center but also centrally for outpatient cases. Events were validated by investigators blinded to estrogen. Cases and controls were interviewed at hospital in a standard way with the same questionnaire. Menopause was defined by amenorrhea for >12 months or bilateral ovariectomy or hysterectomy and age >52 years. Women were classified as current users if they used estrogen at any time in the past 3 months before the case admission date. Hypertension was defined as self-reported systolic pressure >140 mm Hg, diastolic pressure >90 mm Hg, or use of antihypertensives. Diabetes mellitus was defined as a self-reported history of physician-diagnosed diabetes, use of antidiabetics, or both; obesity was defined as a body mass index (BMI) >30 kg/m^2.

Venous blood was obtained from the antecubital vein and anticoagulated with EDTA. DNA was extracted centrally by the salt precipitation method. The presence of factor V Leiden and of the prothrombin G20210A mutation was identified by TaqMan polymerase chain reaction as previously described by Happich et al.[8,9] The protocol was approved by INSERM and the local ethics committee. Written informed consent was obtained from all women.

Statistical Analysis

To determine whether the controls were in Hardy-Weinberg equilibrium, their genotype frequencies were tested with the exact test.[10] Continuous data are expressed as means and SD; qualitative variables are given as absolute values and percentages. Two-tailed Student's *t* test was used to compare parametric variables, and the χ^2 test was used for qualitative variables.

We estimated the relative risks associated with prothrombotic mutations and route of estrogen administration by exposure odds ratios (ORs). The 95% CIs were estimated from the unconditional logistic regression model. The original matching was taken into account by adjustment for age and center. BMI was considered a priori as a potential confounding variable and was included in the multivariate analysis.

There was no association between past use of estrogen and VTE risk, so we pooled past users and never users. In some analyses, we grouped factor V Leiden and prothrombin G20210A carriers together. We investigated the effect of the combination of prothrombotic mutations and estrogen use by comparing those with either risk factor or those with both risk factors with those with neither. Moreover, current users of oral estrogen were compared with current users of transdermal estrogen by carriership of prothrombotic mutations. Interactions between estrogen and prothrombotic mutations were tested by a multiplicative OR model. Statistical analyses were performed with SAS statistical software (version 8.2, SAS Institute Inc).

Results

Two hundred thirty-five cases (128 with PE, 107 with DVT) and 554 controls were successfully genotyped for the factor V Leiden and G20210A mutation. Overall, values were missing for 9% of participants. Most women (95%) were white. General characteristics of patients and controls are shown in Table 1. Mean BMI was higher in patients than in controls.

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TABLE 2. OR of VTE in Relation to the Presence of 1 Prothrombotic Mutation or Estrogen Use

	Patients (n=235), n (%)	Controls (n=554), n (%)	OR (95%CI)	
			Crude	Adjusted*
Estrogen therapy				
Nonuse†	124 (52.8)	341 (61.6)	1	1
Oral estrogen use	51 (21.7)	44 (7.9)	3.2‡ (2.0–5.0)	4.3§ (2.6–7.2)
Transdermal estrogen use	60 (25.5)	169 (30.5)	1.0 (0.7–1.4)	1.2‖ (0.8–1.7)
Factor V Leiden				
GG	200 (85.1)	527 (95.1)	1	1
AG or AA	35 (14.9)	27 (4.9)	3.4‡ (2.0–5.8)	3.2¶ (1.8–5.5)
Prothrombin G20210A				
GG	209 (88.9)	540 (97.5)	1	1
AG or AA	26 (11.1)	14 (2.5)	4.8‡ (2.5–9.4)	4.8¶ (2.6–10.3)

*Adjusted for age, center, and BMI.
†Nonusers include never and past users.
‡$P<0.0001$.
§$P<0.0001$, also adjusted for prothrombotic mutation.
‖P=NS, also adjusted for prothrombotic mutation.
¶$P<0.0001$, also adjusted for oral estrogen use.

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Patients were more likely than controls to have reported a history of varicose veins.

Most current users of estrogen therapy received 17β-estradiol. No controls and only 2 patients used conjugated equine estrogens. Most current users of transdermal estrogen received preparations delivering $\leq$50 μg/d. Less than 10% of transdermal estrogen users received preparations delivering $\geq$100 μg/d. In current users of oral estrogen therapy, the mean dose of estradiol was 1.5 mg/d, ranging from 0.5 mg to 2 mg daily.

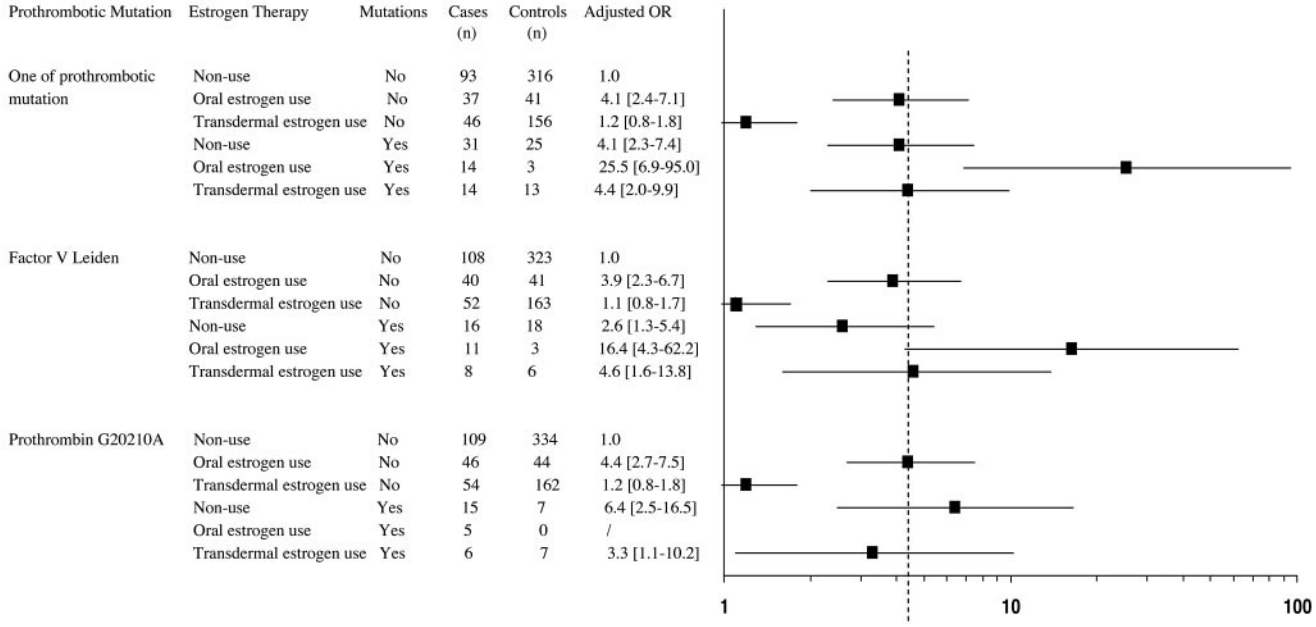
Overall, about a fifth of cases and <10% of controls were current users of oral estrogen, and about a quarter of patients and a third of controls were current users of transdermal estrogen (Table 2). With nonusers as a reference, the OR for VTE was significantly increased in current users of oral estrogen (OR, 3.2; 95% CI, 2.0 to 5.0) but not in current users of transdermal estrogen (OR, 1.0; 95% CI, 0.7 to 1.4). Adjustment for age, center, BMI, and prothrombotic mutations slightly increased these ORs (OR, 4.3; 95% CI, 2.6 to 7.2; and OR, 1.2; 95% CI, 0.8 to 1.7, respectively).

The genotype frequencies of the prothrombotic mutations in controls were in Hardy-Weinberg equilibrium ($P\approx1$ for both prothrombotic mutations). The prevalence of the prothrombotic mutations among controls was 4.9% for the factor V Leiden and 2.5% for the prothrombin G20210A mutation, as expected in a white population (Table 2). Two cases and no controls had both genetic mutations. Factor V Leiden was associated with a 3.4-fold–increased risk of VTE with women without the mutation as a reference. In the same way, prothrombin G20210A mutation was associated with a 4.8-fold–increased risk of VTE. Adjustment for age, center, BMI, and oral estrogen use made little changes in the results.

The Figure shows estimated ORs in relation to the route of estrogen administration and the presence of prothrombotic mutations. Among women without prothrombotic mutation, the adjusted ORs for VTE associated with current use of oral and transdermal estrogen were 4.1 (95% CI, 2.4 to 7.1) and 1.2 (95% CI, 0.8 to 1.8), respectively. The VTE risk associated with the presence of one of the prothrombotic mutations among nonusers was 4.1 (95% CI, 2.3 to 7.4) (the Figure). The combination of current use of oral estrogen and the presence of 1 prothrombotic mutation led to a 25-fold–increased risk of VTE compared with women without a prothrombotic mutation who did not use any treatment (OR, 25.5; 95% CI, 6.9 to 95.0). However, women with a prothrombotic mutation using transdermal estrogen had a risk similar to that of women with a mutation who were not using estrogen (OR, 4.4; 95% CI, 2.0 to 9.9; and OR, 4.1; 95% CI, 2.3 to 7.4, respectively). There was no significant interaction between oral estrogen use and the presence of 1 prothrombotic mutation ($P=0.7$).

Compared with nonusers with factor V Leiden, adjusted ORs for VTE were 6.3 (95% CI, 1.4 to 27.6) and 1.8 (95% CI, 0.5 to 6.3) for current use of oral and transdermal estrogen, respectively. Compared with nonusers with prothrombin G20210A mutation, the adjusted OR for VTE was 0.5 (95% CI, 0.1 to 2.2) for current use of transdermal estrogen. No control both carried prothrombin G20210A mutation and used oral estrogen; thus, the OR for VTE could not be estimated. Two patients who carried both genetic mutations used oral estrogen, but the OR for VTE could not be estimated among these individuals.

Finally, transdermal estrogen did not confer additional risk on women who carried a prothrombotic mutation. In addition, compared with current users of transdermal estrogen, current users of oral estrogen had a significantly increased VTE risk among women with and without prothrombotic mutation (OR, 4.3; 95% CI, 1.0 to 18.6; and OR, 3.1; 95% CI, 1.8 to 5.3, respectively) (Table 3). Further adjustment for potential confounding factors, including smoking, educational level beyond secondary, varicose veins, and cardiovascular risk factors, made little changes in the results.



Risk of VTE by the presence of prothrombotic mutations and estrogen use. Values are OR (95% CI). OR is adjusted for age, center, and BMI. Dotted vertical line indicates the OR of VTE associated with oral estrogen use in the whole population (OR, 4.3; 95% CI, 2.6 to 7.2).

Stratified analyses showed no striking difference in VTE risk according to the type of diagnosis of VTE (DVT versus PE), type of recruitment (in-patient cases versus outpatient cases), or cardiovascular risk factors (women at high risk versus women at low risk). However, the number of cases within subgroups was too small to support adjusted models with reliable estimates for VTE.

Analyses of determinants of the route of estrogen administration showed no striking differences between oral and transdermal estrogen users. On average, women who used

TABLE 3. OR of VTE in Relation to the Presence of Prothrombotic Mutation and Route of Estrogen Administration

Prothrombotic Mutation	Estrogen Therapy	Patients, n	Controls, n	OR (95%CI) Crude	OR (95%CI) Adjusted*
No	Relative to nonusers				
	Nonuse	93	316	1	1
	Oral estrogen use	37	41	3.1[2] (1.9–5.1)	4.1[2] (2.4–7.1)
	Transdermal estrogen use	46	156	1.0 (0.7–1.5)	1.2 (0.8–1.8)
	Relative to transdermal estrogen users				
	Transdermal estrogen use	46	156	1	1
	Oral estrogen use	37	41	3.1‡ (1.8–5.3)	3.5‡ (2.0–6.1)
Yes†	Relative to nonusers				
	Nonuse	31	25	1	1
	Oral estrogen use	14	3	3.8§ (1.0–14.6)	6.2‖ (1.5–25.3)
	Transdermal estrogen use	14	13	0.9 (0.3–2.2)	1.1 (0.4–2.8)
	Relative to transdermal estrogen users				
	Transdermal estrogen use	14	13	1	1
	Oral estrogen use	14	3	4.3§ (1.0–18.6)	5.8‖ (1.3–25.4)

*Adjusted for age, center, and BMI.
†Yes indicates factor V Leiden or prothrombin G20210A.
‡$P<0.0001$.
§$P=0.05$.
‖$P=0.02$.

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transdermal estrogen were 3 years older than those using oral estrogen among controls ($P<0.01$), but there was no significant difference in cardiovascular risk factors between the 2 groups.

Discussion

These data confirm the associations between increased VTE risk and current use of oral estrogen or the presence of prothrombotic mutation. In women who both carry a prothrombotic mutation and use oral estrogen, VTE risk is increased 25-fold compared with nonusers without mutation. In contrast, current use of transdermal estrogen does not confer additional risk on women who carry a prothrombotic mutation.

Prevalence of the factor V Leiden and prothrombin G20210A mutations observed in controls and the increase in VTE risk among women with a prothrombotic mutation are consistent with earlier reports.[4] The joint effect of factor V Leiden or prothrombin G20210A mutation and estrogen use among postmenopausal women was studied previously in 2 case-control studies.[5,6] However, these studies evaluated only the oral route of estrogen administration with limited numbers of cases. Herrington et al[5] included 48 patients and 122 controls, and Rosendaal et al[6,11] included 77 patients and 163 controls. These data showed that both factor V Leiden and oral estrogen use were associated with a substantial increase in VTE risk either independently[5] or with an interaction between these 2 risk factors.[6] Recently, the impact of prothrombotic mutations on the association between estrogen plus progestin and the VTE risk was studied in the WHI trial.[7] In that trial, factor V Leiden, but not the prothrombin G20210A mutation, enhanced the hormone-associated risk of VTE. The statistical power was decreased by the high degree of nonadherence to study medications, however, resulting in an underestimation of hormone effects. In addition, inclusion of procedure-related VTE could also dilute the effects of both hormone therapy and prothrombotic mutations on VTE. In the ESTHER study, VTE cases with predisposing factors were excluded, and only idiopathic events were assessed. Such a selection of patients may explain why our VTE risk estimates are higher than those observed in other studies.[5–7] Our finding of a 25-fold–increased risk of VTE among carriers of prothrombotic mutation using oral estrogen suggests that the effects of both factors are independent. However, the absence of significant interaction may be due to a lack of statistical power. In the ESTHER study, women using transdermal estrogen and carrying a prothrombotic mutation have a VTE risk close to that of nonusers with prothrombotic mutation.

Biological evidence lending support to the difference in VTE risk between oral and transdermal estrogen has been discussed.[2] Oral, not transdermal, estrogen increases plasma concentrations of prothrombin fragment, which is a marker for in vivo thrombin generation, and increases the fibrinolytic potential in postmenopausal women.[12] A lower antithrombin concentration also has been shown in women using oral estrogen but not in those using transdermal estrogen.[13] In addition, an acquired resistance to activated protein C has been demonstrated in users of oral estrogen,[14] but 2 randomized trials recently indicated that these results did not apply to users of transdermal estrogen.[15,16] Thus, oral estrogen may impair the balance between procoagulant factors and antithrombotic mechanisms, whereas transdermal estrogen appears to have little or no effect on hemostasis.

One potential limitation of our study is that observational studies are subject to bias. Possible selection biases among both in-patient cases and hospital controls have been discussed,[2] but such biases should have occurred differentially according to the route of estrogen administration to affect the comparison between oral and transdermal estrogen. Women with recurrent thromboembolism were excluded because a previous episode of VTE would contraindicate hormone therapy. Similarly, cases with predisposing factors for VTE were excluded because these risk factors could determine estrogen use.[17] Outpatient cases were recruited on a consecutive basis after exclusion of women referred to a clinical center for hormone therapy advise or prothrombotic mutation. All diagnoses of VTE required confirmation by imaging methods, and clinical events were validated by investigators blinded to estrogen use and the presence of prothrombotic mutation. The ratio of EP to DVT was higher in hospital cases than in outpatient cases. This difference is probably related to the greater severity of VTE among hospital cases. However, stratified analyses showed no significant difference in VTE risk according to the type of diagnosis. Thus, our results may be generalized more easily to all cases of VTE.

Because PE accounts for about one third of the excess incidence of potentially fatal events with hormone therapy,[7] our findings may have important clinical implications. Women using oral estrogen who carry a prothrombotic mutation can define a subgroup at high VTE risk. It seems that the risk-to-benefit profile of ET is not favorable for these women, even though they suffer menopausal symptoms. Therefore, oral estrogen should be avoided among such women. In contrast, transdermal estrogen may be safe with respect to thrombotic risk, particularly among carriers of a prothrombotic mutation. These data may have potential implications to minimize the excess risk of VTE among women who require hormone therapy. However, the safety of transdermal estrogen has to be confirmed in randomized trials.

In conclusion, the thrombotic risk associated with oral estrogen use is substantially increased among women carrying a prothrombotic mutation. Transdermal estrogen administration seems safer than oral estrogen administration with respect to thrombotic risk, especially among women carrying a prothrombotic mutation. This pattern of association is biologically plausible. The effects of transdermal estrogen among carriers of prothrombotic mutation should be assessed in randomized trials.

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Straczek, Oger, Plu-Bureau, Conard, Barrellier, Meyer, Wahl, Trillot, Alhenc-Gelas, Lévesque, Canonico, and Scarabin collaborated on data acquisition; Drs Straczek, Oger, Plu-Bureau, Canonico, and Scarabin contributed to analysis and interpretation of the data; Drs Straczek, Oger, Plu-Bureau, Conard, Barrellier, Meyer, Wahl, Trillot, Alhenc-Gelas, Lévesque, Canonico, and Scarabin drafted the manuscript; and Drs Straczek, Canonico, and Scarabin were responsible for critical revision of the manuscript for important intellectual content.

Disclosures

None.

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Prothrombotic Mutations, Hormone Therapy, and Venous Thromboembolism Among Postmenopausal Women: Impact of the Route of Estrogen Administration

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