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Marianne Canonico, Elodie Bouaziz, Laure Carcaillon, Céline Verstuyft, Anne Guiochon-Mantel, Laurent Becquemont, and Pierre-Yves Scarabin, for the Estrogen and Thromboembolism Risk (ESTHER) Study Group

Institut National de la Santé et de la Recherche Médicale Unit 780 (M.C., L.C., P.-Y.S.), Cardiovascular Epidemiology Section, and Université Paris-Sud, Institut Fédératif de Recherche 69, Villejuif, F- 94807 France; Laboratoire de Génétique moléculaire, Pharmacogénétique et Hormonologie (E.B., C.V., A.G.-M., L.B.), Hôpital Bicêtre, and Department of Pharmacology (C.V., L.B.), Université Paris-Sud, Le Kremlin-Bicêtre, F-94275 France; and Institut National de la Santé et de la Recherche Médicale Unit 693 (A.G.-M.), "Récepteurs stéroïdiens, Physiopathologie endocrinienne et métabolique", Institut Fédératif de Recherche 93, Université Paris-Sud, Faculté de médecine Paris sud, Le Kremlin-Bicêtre, F-94276 France

Context: Hormone therapy increases the risk of venous thromboembolism (VTE) among postmenopausal women. This effect may be modulated by the expression of cytochromes P450 3A5 (CYP3A5) and 1A2 (CYP1A2) which are involved in the hepatic metabolism of estrogens.

Objective: The objective was to investigate the impact of *CYP3A5* and *CYP1A2* genetic polymorphisms on the association of VTE with hormone therapy.

Design: We conducted a case-control study.

Setting: This study was conducted in eight French hospital centers and in the general population.

Patients: *CYP3A5* and *CYP1A2* genotypes were evaluated among 195 cases with a first documented episode of idiopathic VTE and 533 controls matched for center, age, and admission date.

Outcome Measure: The outcome measure was hormone therapy by route of estrogen administration.

Results: Overall, oral but not transdermal estrogen increased VTE risk [odds ratio (OR) = 4.5, 95% confidence interval (CI) = 2.6–7.6, and OR = 1.2, 95% CI = 0.8–1.8, respectively]. The allele frequency of *CYP3A5*1* was 9 and 10% among cases and controls (OR = 1.0; 95% CI = 0.6–1.5) and that of *CYP1A2*1F* was 72 and 71% among cases and controls (OR = 1.5; 95% CI = 0.8–2.8). Compared with nonusers, OR for VTE in users of oral estrogen was 3.8 (95% CI = 2.1–6.7) among patients without *CYP3A5*1* allele and 30.0 (95% CI = 4.4–202.9) among patients with this allele (test for interaction $P = 0.04$). By contrast, there was no significant interaction between *CYP3A5*1* allele and transdermal estrogen on VTE risk. There is no interaction between *CYP1A2* genetic polymorphism and hormone therapy on VTE risk.

Conclusions: Women with *CYP3A5*1* allele using oral estrogen can define a subgroup at high VTE risk. Additional data are needed to assess the relevance of this genetic biomarker in the medical management of menopause. **(*J Clin Endocrinol Metab* 93: 3082–3087, 2008)**

Oral estrogen therapy is known to increase the risk of venous thromboembolism (VTE) among postmenopausal women (1, 2), whereas recent data suggest that transdermal estrogen use is safe (3, 4). The increased VTE risk among users of oral estrogen is attributed to the coagulation activation due to the hepatic first-pass effect of oral estrogen. Oral but not transdermal es-

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2008-0450 Received February 27, 2008. Accepted May 15, 2008.

First Published Online July 15, 2008

Abbreviations: CI, Confidence interval; CYP, cytochrome P450; ESTHER, Estrogen and Thromboembolism Risk; OR, odds ratio; SNP, single-nucleotide polymorphism; VTE, venous thromboembolism.

trogen induces an increase in the estrone to 17β-estradiol ratio and induces proteins synthesis (5). In the liver, both estrone and 17β-estradiol are catabolized by several hepatic enzymes that catalyze the formation of specific metabolites (6). In particular, the human cytochromes P450 3A5 (CYP3A5) and 1A2 (CYP1A2) are implicated in the hepatic metabolism of estrogens (7). CYP3A5 is mainly expressed in the liver and has a strong genetic basis. Its expression is linked to the single-nucleotide polymorphism (SNP) 6986A→G in intron 3 that causes alternative splicing and a nonfunctional truncated protein (8). Low expression of CYP3A5 is found in homozygous carriers of the *CYP3A5*3* allele, whereas homozygous and heterozygous carriers of the *CYP3A5*1* allele exhibit high expression of CYP3A5 (8). With regard to CYP1A2, several polymorphisms, including the SNP −163C→A in the intron 1 (*CYP1A2*1F*), have been characterized in the past few years (9). Although data on this specific mutation effect are somewhat controversial (10), this polymorphism may be correlated to CYP1A2 inducibility, especially in smokers (11, 12). Whether or not the mutations of *CYP3A5* and *CYP1A2* influence the association of VTE with oral estrogens remains unknown. Therefore, in the ESTHER study, we investigated the impact of the *CYP3A5* and *CYP1A2* genetic polymorphisms on the association between hormone therapy by route of estrogen administration and VTE risk among Caucasian postmenopausal women.

Subjects and Methods

The ESTHER study is a multicenter case-control study aimed to investigate the impact of the route of estrogen administration on VTE risk among postmenopausal women aged 45–70 yr. The study was carried out in France in eight hospital centers and in the general population between 1998 and 2006. A detailed description has been published (3, 4, 13).

Selection of cases and controls

During 1998–2006, 208 hospital cases with a first documented episode of idiopathic VTE were recruited consecutively, and 426 hospital controls were matched by center, 2-yr age band, and admission date. Each case was matched with one to three controls on an individual basis. Hospital controls had to have been admitted in the same hospital with a diagnosis *a priori* unrelated to estrogen use, including diseases of eye, ear, skin, respiratory and alimentary tracts, bones and joints, kidneys, infectious diseases, and diabetes. Moreover, between 1999 and 2002, we consecutively included 63 outpatient cases from three hematology outpatient clinics and their community controls (n = 184) selected at random from electoral rolls and matched by age and area of residence.

Both hospital and outpatient cases were excluded if they reported a personal history of VTE, had a contraindication for hormone therapy (breast cancer, cardiovascular diseases, *etc.*), or had a predisposing factor for VTE (history within the previous month of surgical intervention, trauma with immobilization for longer than 8 d, illness necessitating bed rest for longer than 8 d, known cancer, or systemic inflammatory disease) or a known thrombophilia. Outpatient cases were also excluded if they were referred for estrogen advice after a VTE. Controls were subject to the same exclusion criteria as were cases. The protocol was approved by Institut National de la Santé et de la Recherche Médicale and the local ethics committee. Written and informed consent was obtained from all women.

Ascertainment of cases

Diagnoses of VTE required confirmation by imaging procedures. Pulmonary embolism was defined as the presence of one of the following: helical computed tomography showing at least one intraluminal defect in one segmental pulmonary artery, high probability ventilation/perfusion scan according to criteria from the Prospective Investigation of Pulmonary Embolism Diagnosis, or pulmonary angiography showing a clot. Deep venous thrombosis was diagnosed using compression ultrasonography. Events were adjudicated within each center but also centrally for outpatient cases. Events were validated by investigators blinded to estrogen.

Data collection

Cases and controls were identified without knowledge of estrogen use, and they were interviewed at hospital with a standardized questionnaire. Individual race was defined by self-reporting of parent's ethnicity. Menopause was defined by amenorrhea for more than 12 months or bilateral ovariectomy or hysterectomy and age older than 52 yr. Women were classified as current users of hormone therapy if they reported use of estrogen within the past 3 months before the case admission date. Identification of hormone type was assessed during the direct interview by showing pictures of available hormone therapy. Current smokers were women who had smoked within the 3 months before inclusion. Varicose veins were defined as a reported history of varicose veins or stripping. Family history of VTE was defined as the occurrence of VTE before 65 yr in a first-degree relative. Hypertension was defined as self-reported systolic pressure greater than 140 mm Hg, diastolic pressure greater than 90 mm Hg, or use of antihypertensives. Diabetes mellitus was defined as a self-reported history of physician-diagnosed diabetes or use of antidiabetics or both.

Genotyping analyses

Venous blood was obtained from the antecubital vein and anticoagulated with EDTA. DNA was extracted centrally by the salt precipitation method, and purified genomic DNA was stored at −20 C. Genotyping for allelic variations was performed using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). *CYP1A2*1F* genotyping was performed as previously described (11, 12). Intron 3 of *CYP3A5* gene was amplified using the following primers: forward, 5′-ACC CAG CTT AAC GAA TGC TCT ACT-3′, and reverse, 5′-GAA GGG TAA TGT GGT CCA AAC AG-3′. The following fluorescent probes (VIC and FAM) were used: VIC, 5′-TTT TGT CTT TCA ATA TCT CTT-3′ for detecting *CYP3A5*1* allele, and FAM, 5′-TTG TCT TTC AGT ATC TCT T-3′ for detecting *CYP3A5*3* allele. Amplification was performed in a final volume of 15 μl containing TaqMan Universal PCR Master Mix, 200 nM of each probe and 900 mM of each primer. The PCR program was started with 2 min at 50 C, 10 min at 95 C, followed by 35 cycles consisting of 92 C for 15 sec and 60 C for 1 min. The post-PCR-generated fluorescence intensity was quantified using an ABI 7000 Sequence Detector System software version 1.2.3 (Applied Biosystems, Courtaboeuf, France). Each SNP genotyping procedure was performed in duplicate (separate experiments) for each patient. Sequenced wild-type homozygous, heterozygous, or muted homozygous patient samples were used as controls. All PCR reagents were purchased from Applied Biosystems (Courtaboeuf, France). Finally, genotypes were obtained for 195 cases (109 with pulmonary embolism and 86 with deep venous thrombosis) and 533 controls. This genetic investigation was a designed *post hoc* study.

Statistical analysis

Continuous data are expressed as means and SD, and qualitative variables are given as absolute values and percentages. Two-tailed Student's *t* test was used for comparison of continuous variables, and χ^2 test for qualitative variables. The χ^2 test was used to compare the observed numbers of each genotype with those expected under Hardy-Weinberg equilibrium.

Crude odds ratios (OR) and 95% confidence intervals (CI) were estimated using an unconditional logistic regression. The original matching was taken into account by adjustment for age, center, and admission date. Further adjustments included potential confounding variables such as obesity, family history of VTE, and varicose veins.

First, we estimated the VTE risk associated with hormone therapy by route of estrogen administration and with genetic mutations of *CYP3A5* and *CYP1A2*. Second, stratified analyses were performed to estimate the impact of hormone therapy by the route of estrogen administration and *CYP3A5* genetic status. Finally, we estimated the VTE risk in relation to *CYP3A5* genetic status and hormone therapy by route of estrogen administration using the nonusers of hormone therapy with *CYP3A5*3/*3* genotype as the reference group. Interactions between hormone therapy and genetic status were tested using a multiplicative OR model.

Statistical analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC).

Results

General characteristics of cases and controls and distribution of *CYP3A5* and *CYP1A2* genotypes are given in Table 1. One hundred ninety-five cases (164 hospital and 31 outpatient cases) and 533 controls (353 hospital and 180 community controls) were successfully genotyped for one or both studied genetic polymorphisms. Women were exclusively Caucasians. Mean body mass index was higher in cases than in controls, and obesity was more frequent among cases than controls. Cases were more likely than controls to have reported a family history of VTE, personal history of varicose veins, and use of oral estrogen therapy.

Most current users of estrogen therapy received 17β-estradiol; no controls and only two cases used conjugated equine estrogens. Regarding transdermal estrogen users, more than 95% of women received preparations delivering 50 μg 17β-estradiol per day or less. Transdermal estrogen was used alone or combined with either micronized progesterone, pregnane derivatives, or norpregnane derivatives. In current users of oral estrogen therapy, the mean dose of estradiol was 1.5 mg/d, ranging from 0.5–2 mg daily. Oral estrogen was administrated alone or combined with micronized progesterone, pregnane derivatives, norpregnane derivatives, or nortestosterone derivatives.

The distributions of *CYP3A5* and *CYP1A2* genotypes in the control subjects were consistent with those predicted under the conditions of Hardy-Weinberg equilibrium ($P > 0.05$ for *CYP3A5* and *CYP1A2*). The frequencies of the *CYP3A5*1* allele and *CYP1A2*1F* allele among controls (9.2 and 70.8%, respectively) were close to those expected in the Caucasian population.

Table 2 shows the OR of VTE by hormone therapy or genetic status. Overall, oral but not transdermal estrogen increased VTE risk (OR = 4.5, 95% CI = 2.6–7.6 and OR = 1.2, 95% CI = 0.8–1.8, respectively, after adjustment for age, center, admission date, obesity, and family history of VTE and varicose veins). Neither *CYP3A5*3* allele nor *CYP1A2*1F* allele was significantly associated with VTE risk (OR = 1.0, 95% CI = 0.6–1.5 and OR = 1.5, 95% CI = 0.8–2.8, respectively, after complete adjustment). With respect to CYP1A2, stratified analyses by smoking status did not change the results (data not shown).

Table 3 shows the VTE risks in relation to hormone therapy (by route of estrogen administration) stratified by *CYP3A5* genotype status. Compared with nonusers, OR for VTE in current users of oral estrogen was 3.8 (95% CI = 2.1–6.7) among patients without the *CYP3A5*1* allele and 30.0 (95% CI = 4.4–202.9) among women who carry at least one copy of *CYP3A5*1* allele. The interaction between *CYP3A5*1* allele and oral estrogen on VTE risk was significant ($P = 0.048$). By contrast, there was no significant interaction between *CYP3A5*1* allele and transdermal estrogen use on VTE risk (OR = 1.0, 95% CI = 0.1–1.7 among patients without *CYP3A5*1* allele and OR = 3.2, 95% CI = 0.9–9.6 among patients with *CYP3A5*1* allele). Stratified analyses by both genotype status of *CYP1A2* and smoking status showed no significant difference in VTE risk associated with hormone therapy by route of estrogen administration (data not shown).

TABLE 1. Characteristics of cases and controls

Variables	Cases (n = 195)	Controls (n = 533)	*P* value
Age (yr)	61.5 (6.64)	61.4 (6.62)	0.986
Body-mass index (kg/m²)	26.9 (5.67)	24.6 (4.65)	<0.0001
Obesity (body mass index >30 kg/m²)	40 (20.5)	65 (12.2)	0.011
Family history of VTE (n)	57 (29.2)	115 (21.6)	0.019
Personal history of varicose veins (n)	109 (55.9)	243 (45.6)	0.011
Estrogen therapy use (n)			<0.0001
Nonusers	101 (51.8)	328 (61.4)	
Current users of oral estrogen	43 (22.1)	43 (8.1)	
Current users of transdermal estrogen	51 (26.1)	162 (30.4)	
CYP3A5 genotype (n)			0.888
*CYP3A5*3/*3*	163 (83.6)	443 (83.1)	
*CYP3A3*3/*1*	30 (15.4)	82 (15.4)	
*CYP3A5*1/*1*	2 (1.0)	8 (1.5)	
CYP1A2 genotype[a] (n)			0.615
*CYP1A2*1A/*1A*	14 (7.2)	49 (9.3)	
*CYP1A2*1A/*1F*	83 (42.6)	211 (39.9)	
*CYP1A2*1F/*1F*	98 (50.2)	269 (50.8)	

Data are number (percent) or mean (SD).

[a] Data for 4 controls are missing.

TABLE 2. OR of VTE by hormone therapy use or genetic mutations (*CYP3A5*1* allele and *CYP1A2*1F* allele)

Variables	Cases (n = 195)	Controls (n = 533)	OR (95% CI)		
			Crude	Adjusted[a]	Adjusted[b]
Hormone therapy					
Non-use	101	328	1	1	1
Oral estrogen use	43	43	3.2 (2.0–5.2)	4.0 (2.4–6.8)	4.5 (2.6–7.6)
Transdermal estrogen use	51	162	1.0 (0.7–1.5)	1.2 (0.8–1.8)	1.2 (0.8–1.8)
CYP3A5 genotype					
*CYP3A5*3/*3*	163	443	1	1	1
*CYP3A5*3/*1 or CYP3A5*1/*1*	32	90	1.0 (0.6–1.5)	1.0 (0.6–1.5)	1.0 (0.6–1.5)
CYP1A2 genotype					
*CYP1A2 *1A/*1A*	14	49	1	1	1
*CYP1A2*1A/*1F or CYP1A2*1F/*1F*	181	480	1.3 (0.7–2.4)	1.4 (0.7–2.6)	1.5 (0.8–2.8)

[a] Adjusted for age, center, and date index.

[b] Further adjusted for obesity and family history of VTE and varicose veins.

Finally, Fig. 1 displays the VTE risk according to *CYP3A5* genotype status and hormone therapy by route of estrogen administration using the nonusers without the *CYP3A5*1* allele as the reference group. Women with the *CYP3A5*1* allele treated by oral estrogen defined a subgroup at high VTE risk.

Discussion

This report shows a synergism between *CYP3A5*1* allele and oral estrogen use on VTE risk. Oral estrogen users who carry *CYP3A5*1* allele can define a subgroup at high VTE risk. By contrast, there is no interaction between *CYP3A5*1* allele and transdermal estrogen use on VTE risk. On the other hand, there is no interaction between *CYP1A2* genetic polymorphism and hormone therapy on VTE risk.

To our knowledge, the present study is the first to investigate the influence of *CYP3A5* genetic polymorphism on the relation between hormone therapy and VTE risk. Previous studies have investigated the influence of *CYP3A5* genetic polymorphisms in other conditions. First, the impact of this mutation on the metabolism of several drugs has been studied namely in solid-organ transplantation. *CYP3A5* is involved in tacrolimus metabolism and modulates its oral disposition. Allograft recipients carrying the *CYP3A5*1* allele need higher daily doses of tacrolimus to achieve plasma target concentrations because of an increased hepatic and intestinal metabolism (14, 15). Second, the *CYP3A5* genetic polymorphism has been investigated in relation to blood pressure. Despite conflicting results, *CYP3A5*1* allele emerged as a risk factor for hypertension (16–19). The mechanism underlying this association could be based on the sodium reabsorption increase among carriers of *CYP3A5*1* allele, possibly via cortisol and/or aldosterone metabolism (16). Finally, *CYP3A5* is involved in estrogen metabolism (7), and *CYP3A5* genetic polymorphism has been studied in estrogen-dependent diseases, especially breast cancer. *CYP3A5* SNP may be implicated in a substantial increase in tamoxifen metabolism and thus leads to a lower efficacy of this therapy on the survival of patients with estrogen-receptor-positive tumors. So, women who did not carry the *CYP3A5*1* allele were more likely to have a recurrence-free survival with prolonged tamoxifen treatment (20).

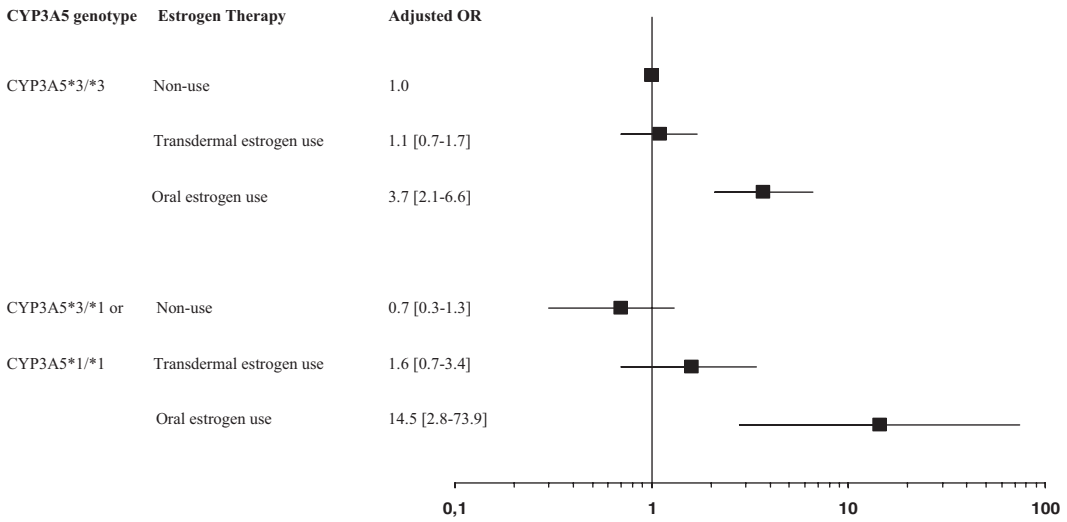
The impact of the *CYP3A5*3* SNP on the relation between oral estrogen use and VTE risk is biologically plausible. When administrated by oral route, estrogen therapy results in a substantial increase in plasma estrone concentration leading to a nonphysiological ratio of estrone to estradiol close to 5. By contrast, transdermal estrogen leads to plasma estrone to estradiol ratios close to 1, which is similar to that in menstruating women and does not alter liver protein production (5, 21). In the liver, there is a dynamic mutual conversion system between estradiol and estrone, and both these estrogens are metabolized by cytochrome P450 (22). Estrogen metabolism can lead to a deactivation and elimination of parent hormone or, alternatively, may result in the production of metabolites that have altered hor-

TABLE 3. OR of VTE in relation to hormone therapy by genotype status of *CYP3A5*

Variables	Cases (n = 195)	Controls (n = 533)	OR (95% CI)		
			Crude	Adjusted[a]	Adjusted[b]
*CYP3A5*3/*3*					
Non-use	88	269	1	1	1
Oral estrogen use	36	41	2.7 (1.6–4.5)	3.5 (2.0–6.1)	3.8 (2.1–6.7)
Transdermal estrogen use	39	133	0.9 (0.6–1.4)	1.1 (0.7–1.7)	1.0 (0.7–1.7)
*CYP3A5*3/*1* or *CYP3A5*1/*1*					
Non-use	13	59	1	1	1
Oral estrogen use	7	2	15.9 (3.0–85.4)	24.9 (4.0–156.1)	30.0 (4.4–202.9)
Transdermal estrogen use	12	29	1.9 (0.8–4.6)	2.6 (0.9–6.9)	3.2 (0.9–9.6)

[a] Adjusted for age, center, and date index.

[b] Further adjusted for obesity and family history of VTE and varicose veins.



Test for interaction between oral estrogen and CYP3A5*1 allele was significant (p=0.0486)
Test for interaction between transdermal estrogen and CYP3A5*1 allele was not significant (p=0.110)

FIG. 1. Risk of VTE in relation to hormone therapy by route of estrogen administration and CYP3A5 genetic status. Values are OR [95% CI] adjusted for age, center, admission date, obesity, and family history of VTE and varicose veins.

monal properties (23). Estrogen metabolism by *CYP3A5* leads in particular to 4-hydroxy derivatives (24) and to 16α-hydroxy derivatives (25). These metabolites could be involved in a more important hormone-responsive process as a result of changes in both the strength of estrogen receptor binding and the dissociation rate of the metabolite-receptor complex (26–29). Because it is now well known that an enhanced estrogenic exposure is an important determinant of VTE risk (3, 30, 31), oral estrogen users who carry the *CYP3A5*1* allele might be at higher risk for VTE.

One potential limitation of our study is that observational studies are subject to bias. The validity of the ESTHER study has already been discussed (3, 4, 13). In the present analysis, potential biases include confounding factors, missing values, and misrepresentation of genotypic distribution. First, elevated levels of VTE risk factors, including age, obesity, and family history of VTE, varicose veins, and prothrombotic mutations (either factor V Leiden or G20210A prothrombin mutation) could explain our findings related to oral estrogen use by *CYP3A5* genetic polymorphism. However, the proportion of women at high VTE risk was similar among oral estrogen users who carry the *CYP3A5*1* allele compared with other different subgroups. Furthermore, adjustment for these potential confounding factors made little change to the results, and there was no interaction of CYP3A5 genetic polymorphism with the prothrombotic mutations, especially factor V Leiden. In addition, the characteristics of hormone therapy in this subgroup at high VTE risk did not significantly differ from the other ones, especially regarding the daily estrogen doses. Second, DNA was not available for 76 cases (28.0%) and for 77 controls (12.6%), and these missing data could result in a bias. However, this bias would have to have occurred differentially according to the hormone therapy use and to the genetic status to affect the results. Finally, another bias could be related to a misrepresentation of genotypic frequencies in our sample. However, the allelic frequency of the *CYP3A5*1* allele among controls was not significantly different according to hormonal therapy.

Our findings could have clinical implications in the medical management of menopause. Pulmonary embolism accounts for about one third of the excess incidence of potentially fatal events due to hormone therapy (30). Recent guidelines now recommend that women are prescribed the lowest effective dose of hormone therapy for the shortest time possible (31). Whereas there is little increased risk of stroke and breast cancer within the first year of treatment, pulmonary embolism has become the major potentially fatal event due to short-term hormone therapy use. Therefore, detection of women at high risk is important in assessing the benefit/risk profile of hormone therapy. Our study shows that the increase in VTE risk in oral estrogen users is more pronounced among women who carry the *CYP3A5*1* allele. By contrast, transdermal estrogen therapy may be safe with respect to thrombotic risk and does not interact with *CYP3A5* genetic polymorphism. These findings could provide additional information for VTE risk stratification among women who require hormone therapy. However, these data need to be confirmed, and performance characteristics of this genetic biomarker, including cost-effectiveness analyze, should be assessed. Further research could also include an *in vitro* determination of the effects of different estrogen metabolites by CYP3A5. In addition, the effect of transdermal estrogen use on VTE risk should to be assessed in randomized controlled trials.

Acknowledgments

Address all correspondence and requests for reprints to: Marianne Canonico, Institut National de la Santé et de la Recherche Médicale (INSERM) Unit 780 (U780), Cardiovascular Epidemiology Section, 16 avenue Paul Vaillant Couturier, Villejuif Cedex, F- 94807 France. E-mail: marianne.canonico@inserm.fr.

This work was supported by the Fondation de France, by the Fondation pour la Recherche Médicale (FRM) and INSERM, and by grants from Aventis, Besins International, Sanofi, and Servier Institute.

P.-Y. Scarabin was the principal investigator and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study

concept and design were the responsibility of P.-Y. Scarabin, E. Oger, and G. Plu-Bureau; acquisition of data was the responsibility of E. Oger, G. Plu-Bureau, J. Conard, M. T. Barrellier, G. Meyer, D. Wahl, N. Trillot, H. Lévesque, C. Verstuyft, L. Carcaillon, E. Bouaziz, L. Becquemont, A. Guiochon-Mantel, M. Canonico, and P.-Y. Scarabin; analysis and interpretation of data were the responsibility of C. Verstuyft, L. Carcaillon, E. Bouaziz, L. Becquemont, A. Guiochon-Mantel, M. Canonico, and P.-Y. Scarabin; and drafting of the manuscript was the responsibility of C. Verstuyft, L. Carcaillon, E. Bouaziz, L. Becquemont, A. Guiochon-Mantel, M. Canonico, and P.-Y. Scarabin.

Clinical centers included Département de Médecine Interne, Hôpital de la Cavale Blanche, Brest (E. Oger and D. Mottier); Service d'Exploration Fonctionnelle, Centre Hospitalier de Caen (M. T. Barrellier); Unité Hémostase-Thrombose, Hôtel-Dieu, Paris (J. Conard and G. Plu-Bureau); Service de Médecine Interne et Médecine Générale, Service d'Hématologie Biologique, Centre Hospitalier et Universitaire de Nancy (D. Wahl, T. Lecompte, and E. de Maistre); Département de Pneumologie, Hôpital Européen Georges Pompidou, Paris (G. Meyer and M. T. Guillanneuf); Laboratoire d'Hématologie, Hôpital Cardiologique, Lille (N. Trillot and B. Jude); Département de Médecine Interne, Centre Hospitalier de Rouen (H. Lévesque and N. Cailleux); and Département de Médecine Vasculaire, Hôpital Purpan, Toulouse (P. Léger and A. Elias).

DNA analysis was done at the Laboratory of Molecular Genetics, Pharmacogenetics, and Hormonology and INSERM U693, "Récepteurs stéroïdiens, Physiopathologie endocrinienne et métabolique" (C.V., E.B., L.B., and A.G.-M.).

The coordinating center was INSERM U780 (G. Plu-Bureau, E. Oger, L. Carcaillon, M. Canonico and P.-Y. Scarabin).

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Disclosure Statement: The authors have nothing to disclose.

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