Does the progesterone receptor genetic polymorphism +331G/A hPR influence the risk of venous thromboembolism among postmenopausal women using hormone therapy? The ESTHER Study.

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Abbreviated title: PR polymorphism, thromboembolism risk and hormone therapy.

Key terms: progesterone receptor, venous thromboembolism, hormone therapy

Word count: 1000

Table: 1

References: 5

Conflict of interest: no.

Funding/Support

The study was supported by the Fondation de France, by the Fondation pour la Recherche Médicale (FRM) and Institut National de la Santé et de la Recherche Médicale (Inserm), and by grants from Aventis, Besins International, Sanofi, and Servier Institute.

Abstract.

Hormone therapy (HT) increases venous thromboembolism (VTE) risk among postmenopausal women. Data on the influence of steroids receptors polymorphisms on this association remain scarce. Since progesterone receptor (hPR) is expressed in human veins and specific progestogens increase VTE risk, we investigated the impact of the functional *+331G/A hPR* polymorphism on the association of VTE with HT. Using the data of the ESTHER study, we showed that ORs for VTE in current users of progesterone or progestins were not significantly different by *hPR +331G/A* genotype status. *hPR* polymorphism appears not to have a significant effect on VTE risk related to HT.

Introduction

Progesterone or progestins are systematically used in combination with estrogen among non-hysterectomised women who require hormone therapy (HT). Cardiovascular disease, including venous thromboembolism (VTE), is an important determinant of the benefit-to-risk profile of HT. Both observational studies and clinical trials have shown a significant increase in VTE risk among postmenopausal women using HT [1]. While the route of estrogen administration represents a major determinant of VTE risk among HT users, recent findings have suggested a differential impact of the progestogen subgroups on thrombotic risk [2]. Elevated risk among postmenopausal women using HT could be due to venous stasis caused by changes in venous distensibility and capacitance induced by progestogens. These effects can be mediated by human nuclear progesterone receptor (hPR) since it has been found in endothelial and smooth muscle cells of the vessel walls [3]. hPR is a member of the steroid-receptor superfamily. Its gene uses two separate promoters to produce two isoforms, hPR-A and hPR-B. hPR-B, which presents an additional 164 aminoacids in its N-terminus domain, is a more potent transactivator than hPR-A. The balance hPR-B to hPR-A is involved in several pathophysiological actions of progesterone [4]. One promoter region polymorphism, *+331G/A hPR*, creates a transcription start site. The *+331A hPR* produces a greater amount of hPR-B protein than *+331G hPR* in endometrial cell lines thus increasing the ratio hPR-B/hPR-A [5]. This functional polymorphism is associated with a risk of endometrial cancer [5]. Whether the balance hPR-B/hPR-A influences the association of VTE with progestogens remains unknown. Therefore, we used the data from the EStrogen and THromboEmbolism Risk (ESTHER) Study to investigate the impact of the *+331G/A hPR* polymorphism on the association between progestogens and VTE.

Subjects and methods

Subjects

The ESTHER study has been previously described [2]. Briefly, it was a multicenter case-control study of VTE performed in France between 1998 and 2006. The population consisted of postmenopausal women aged 45 to 70 years with neither a personal history of VTE nor contra-indication to HT, nor predisposing factors for VTE. Cases with a first documented episode of idiopathic VTE (n=271) were matched to one to three controls (n=610) for age, center and admission date. The protocol was approved by INSERM and the local ethics committee. Written and informed consent was obtained from all women.

hPR genotyping

+331G/A hPR genotyping was performed on genomic DNA by a real-time polymerase chain reaction TaqMan allelic discrimination assay, designed using Primer Express Software (Applied Biosystems) with primers previously described [5]. Homozygous *+331A/A hPR* alleles were confirmed by genomic sequencing.

Statistical methods

To determine whether the controls were in Hardy-Weinberg equilibrium, their genotype frequencies were tested with the chi-square test. 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. As previously described, current users of HT were compared with non-users in a joint model of both route of estrogen administration and progestogens types [2]. Interactions between HT and *+331G/A hPR* genetic status (either *G/G* or *G/A+A/A)* were tested by using a multiplicative OR model. Statistical analyses were performed using SAS statistical software (version 9.1, SAS Institute Inc, Cary, NC).

Results

A total of 195 DNA cases and 519 DNA controls were available and successfully genotyped for the *+331G/A hPR* polymorphism. The observed allele frequencies did not deviate from the expected Hardy-Weinberg distribution in our population and allele frequencies were in the same range to those described in Caucasian population (*+331A hPR*: 0.017, *+331G hPR*: 0.983).

The variant *+331A hPR* allele frequency was 6.2% and 4.8% among cases and controls respectively (OR=1.4; 95% CI: 0.9-2.5). Compared with non-users, OR of VTE in current users of oral and transdermal estrogens were 3.8 (1.5-9.9) and 1.1 (0.5-2.4) respectively among patients with *+331G/G hPR* genotype and 18.2 (0.4-923.6) and 0.8 (0.1-9.8) respectively among carriers of *+331A hPR* allele. Regarding the impact of progestogens, the OR of VTE in current users of micronised progesterone or pregnanes and norpregnanes were 0.8 (0.4-1.9) and 2.5 (1.1-6.3) respectively for women without the A allele and 0.3 (0.1-6.8) and 6.5 (0.3-166.2) respectively for carriers of the *+331A hPR* allele. Tests for interaction between HT and hPR status on VTE risk were not significant (Table 1). Furthermore, there was no interaction of *+331G/A hPR* genetic polymorphism with body-mass index (BMI) on VTE risk (data not shown).

Discussion

To our knowledge, this study is the first to investigate the impact of *+331G/A hPR* genetic polymorphism on VTE risk. The present analysis confirms that micronised progesterone and pregnanes are not associated with an increased VTE risk whereas norpregnanes may be thrombogenic. In addition, our results show that the *+331G/A hPR* polymorphism has no significant influence on VTE risk among postmenopausal women using HT.

Our results suggest that the *+331G/A hPR* polymorphism may be not functional in endothelial and smooth muscle cells of the vessel wall. Indeed, its effect has only been studied *in vitro* in endometrial cancer cells [5]. Alternatively, they suggest that there is no interaction between nuclear hPR and VTE and that the progestogens thrombotic effects are non genomic. At last, these effects may be mediated not only by hPR but also by other steroid receptors, such as androgen receptor.

As BMI is also a VTE risk factor and the interaction between BMI and the *+331G/A hPR* polymorphism has previously been described [5] we tested this interaction in our population. Interestingly, there was no interaction of *+331G/A hPR* genetic polymorphism with BMI on VTE risk.

The validity of the ESTHER study has been discussed [2]. In this analysis, the main limitation is the low sample size of subgroups and the limited statistical power to detect an interaction between HT and hPR genetic status. Of particular interest is the population stratification in genetic investigations [2].

In conclusion, further studies are needed to investigate the influence of hPR polymorphism, as well as other steroid receptors, and non genomic effects in the association of VTE with HT.

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Conflict of interest: no.

Funding/Support

The study was supported by the Fondation de France, by the Fondation pour la Recherche Médicale (FRM) and Institut National de la Santé et de la Recherche Médicale (Inserm), and by grants from Aventis, Besins International, Sanofi, and Servier Institute.

Role of sponsors

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

Acknowledgements: We are grateful to L. Dubert for her precious technical assistance and Pr. P. Jaillon from the department of Pharmacology of St Antoine Hospital, University Paris 6.

Table 1: Odds ratio for VTE in relation to HT by genotype status of *hPR +331G/A*

hPR status	Hormone therapy	Cases (n=187)	Controls (n=513)	Odds ratios (95% CI)*
GG	Non-use	88	292	1
	Oral estrogen	30	32	3.8 (1.5-9.9)
	Transdermal estrogen	46	142	1.1 (0.5-2.4)
	No progestogen	11	33	-
	Micronised progesterone + Pregnanes	41	110	0.8 (0.4-1.9)
	Norpregnanes	24	31	2.5 (1.1-6.3)
GA or AA	Non-use	13	27	1
	Oral estrogen	5	2	18.2 (0.4-923.6)
	Transdermal estrogen	5	18	0.8 (0.1-9.8)
	No progestogen	1	3	-
	Micronised progesterone + Pregnanes	4	14	0.3 (0.1-6.8)
	Norpregnanes	5	3	6.5 (0.3-166.2)

Users of oral estrogen combined with nortestosterone derivatives were excluded and OR for VTE were estimated separately using the non-users as the reference group (7 cases and 6 controls among patients with h*PR +331G/G* genotype [OR=4.0; 95% CI: 1.3-12.6] and 1 case and 0 control among carriers of h*PR +331A* allele [OR not available]).

* Adjusted for age, center, admission date, family history of VTE, history of varicose veins and obesity
Test for interaction between oral estrogen use and A allele was not significant (p=0.46)
Test for interaction between transdermal estrogen use and A allele was not significant (p=0.70)
Test for interaction between micronised progesterone + pregnanes use and A allele was not significant (p=0.51)
Test for interaction between norpregnanes use and A allele was not significant (p=0.63)