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Genetic risk factors for venous thrombosis in women using combined oral contraceptives: update of the PILGRIM study

P Suchon, F Al Frouh, M Ibrahim, G Sarlon, G Venton, MC Alessi, DA Trégouët, PE Morange

Abstract

Identifying women at risk of venous thrombosis (VT) under combined oral contraceptives (COC) is a major public health issue. The aim of this study was to investigate in COC users the impact on disease of genetic polymorphisms recently identified to associate with VT risk in the general population. Nine polymorphisms located on *KNG1*, *F11*, *F5*, *F2*, *PROCR*, *FGG*, *TSPAN* and *SLC44A2* genes were genotyped in a sample of 766 patients and 464 controls as part of the PILGRIM (PIL Genetic Risk Monitoring) study. Cases were women who experienced an episode of documented VT during COC use, while controls were women with no history of VT using COC at the time of inclusion. Among the studied polymorphisms, only *F11* rs2289252 was significantly associated with VT. The *F11* rs2289252-A allele was associated with a 1.6-fold increased risk of VT ($p < 0.0001$). Besides, the combination of the rs2289252-A allele with non-O blood group, present in 52% of the cohort, was associated with an odds ratio of 4.00 (2.49–6.47; $p < 10^{-4}$). The consideration of this genetic risk factor could help to better assess the risk of VT in COC users.

In women, the overall incidence of venous thrombosis (VT) is about 1.2 per 1000 person-years¹; this incidence being lower in women of reproductive age, with an estimate ranging between 0.4 and 0.6 per 1000 person-years¹. The use of combined oral contraceptive (COC) is a major established risk factor for VT in women of reproductive age. The relative risk for COC use is about 3–6. It is well known that this risk depends on two major factors: the progestogen and the ethinyl estradiol dosage². It is estimated that more than 100 million women worldwide are using COC, making the impact of COC use on VT risk and public health of major importance³. In France, 20 young women die annually of pulmonary embolism (PE) as a consequence of COC use⁴. Furthermore, young and middle-aged women with a personal history of VT have a 2.3-fold increased risk of mortality⁵. Besides, among survivors, 25–50% will have lasting debilitating health problems such as post-thrombotic syndrome, severely hampering mobility and quality of life⁶. Chronic thromboembolic pulmonary hypertension occurs in about 3% PE patients⁷.

Oral contraceptives and inherited thrombophilic defects (i.e. factor V Leiden and prothrombin mutations, deficiencies of protein C, protein S or antithrombin) are known to interact synergistically to increase the risk of VT⁸. Very recently, we have found that the ABO blood group, an increasingly recognized risk factor for VT in the general population, was also associated with the risk of VT in COC users. In the PILGRIM (Pill Genetic Risk Monitoring) study, we observed that, under COC use, women of non-O blood group displayed a 1.85-fold increased risk of VT compared with O blood group carriers⁹.

Last year, the International VENous Thrombosis (INVENT) consortium presented the results of the first international meta-analysis of genome-wide association studies for VT in the general population¹⁰. This project led to the identification of two unsuspected susceptibility

loci for VT (*TSPAN15* and *SLC44A2*) and confirmed the strong associations with VT of several previously reported genes (*F5*, *F2*, *FGG*, *F11* and *PROCR*). The aim of this study was to assess the impact of the lead common single nucleotide polymorphisms (SNPs) of these VT-associated genes on VT risk in COC users.

Materials and methods

PILGRIM is a case–control study including child-bearing age women referred to a single center [Exploration Centre of hemorrhagic and thrombotic diseases (CEHT) of the Marseille University Hospital] between 1 January 2003 and 31 December 2013. Cases were all consecutive women who had a first objectively confirmed (i.e. evidenced by imaging or when the patient had received a full-dose anticoagulant therapy for at least 3 months) episode of VT (including PE and proximal or distal deep vein thrombosis of the lower limbs) during COC use, with no history of cancer or autoimmune disease. Controls were defined as consecutive women using COC, who were referred to CEHT for thrombophilia screening as they presented a family history of thrombosis but with no personal history of the disease. Women using first generation pills were excluded. Pills were categorized according to the generation of the progestogen. The whole PILGRIM cohort comprises 968 cases and 874 controls and is extensively described by Suchon et al. 9

Stored DNA was available in 766 (79.1%) cases and 464 (53.1%) controls who participated in this study. Although there are fewer individuals in this study, their general characteristics were comparable to those of the whole PILGRIM study. These PILGRIM participants were genotyped for nine SNPs reported by the INVENT consortium to associate with VT risk, including the lead SNPs at two novel susceptibility genes, *SLC44A2* rs2288904 and *TSPAN15* rs78707713, and seven SNPs at known VT-associated genes that achieved strong statistical association, *F5* rs4524, *F2* rs3136516, *KNG1* rs710446, *F11* rs2289252, *F11* rs2036914, *FGG* rs2066865 and *PROCR* rs867186 10. Genotyping was performed using real-time polymerase chain reaction.

For each studied SNP, deviation from Hardy–Weinberg model was tested in controls using a chi-squared test with one degree of freedom. Association with VT risk was tested by use of the Cochran–Armitage trend test. Allelic odds ratios (ORs) and 95% confidence intervals (CIs) were also computed using logistic regression analysis, adjusting for age, type and duration of COC use, and family history score (calculated by dividing the number of first-degree relatives with a personal history of VT by the total number of first-degree relatives) 9. A statistical threshold of 6×10^{-3} ($\sim 0.05/9$), correcting for the number of tested polymorphisms, was used to declare statistical significance for the observed SNP association with disease risk. All statistical analyses were performed using R.

We obtained the informed consent from all participants, and the study met all institutional ethics requirement. The procedures employed were reviewed and approved by the Assistance Publique des Hopitaux de Marseille institutional review committee.

Results

Main characteristics of the population study are shown in Table 1 and are comparable to the characteristics of the whole PILGRIM cohort previously reported. In particular, the four main risk factors for VT risk we previously identified in the whole PILGRIM study 9, which were smoking, body mass index (BMI) greater than 35 kg/m² (class II obesity and above according

to the World Health Organization classification of obesity), severe inherited thrombophilia and non-O blood groups, were consistently associated with the disease in the current PILGRIM participants. The corresponding ORs for VT were 1.65 [95% CI (1.30–2.10)], 3.46 (1.81–7.03), 2.13 (1.32–3.51) and 1.98 (1.57–2.49), respectively (data not shown).

Table 1. Main characteristics of the population study^a

	VT patients (<i>n</i> = 766)	Controls (<i>n</i> = 464)	<i>p</i> value
Mean (SD) age (years)	32.2 (9.5)	30.5 (8.8)	0.0008
Oral contraceptive use by generation			
Second ^b	262 (34.2)	173 (37.3)	
Third ^c	315 (41.1)	135 (29.1)	<10 ⁻⁴
Fourth ^d	96 (12.5)	70 (15.1)	
Cyproterone acetate	93 (12.1)	86 (18.5)	
Mean duration of COC in years (SD) ^e	8.4 (7.4)	7.5 (5.5)	0.02
Duration of use ≤12 months	106 (14.8)	20 (4.7)	<10 ⁻⁴
Positive first-degree family history of VT ^f	213 (27.8)	271 (58.4)	<10 ⁻⁴
Mean family history score (SD)	0.07 (0.13)	0.17 (0.17)	<10 ⁻⁴
Mean (SD) body mass index (BMI) ^g	23.9 (5.1)	22.1 (3.7)	<10 ⁻⁴
BMI ≥ 30 kg/m ²	95 (12.5)	18 (4.0)	<10 ⁻⁴
BMI ≥ 35 kg/m ²	36 (4.7)	5 (1.1)	0.0007
Current smokers ^h	254 (33.2)	103 (22.2)	<10 ⁻⁴
Severe thrombophilia ⁱ	52 (6.8)	22 (4.7)	0.14
ABO blood group			
A	440 (57.4)	229 (49.4)	
B	93 (12.1)	46 (9.9)	<10 ⁻⁴
AB	54 (7.0)	22 (4.7)	
O	179 (23.4)	167 (36.0)	
Non-O	587 (76.6)	297 (64.0)	<10 ⁻⁴

- COC, combined oral contraceptives; VT, venous thrombosis.
- a Shown data are counts (%) unless otherwise indicated.
- b Second generation: levonorgestrel, norgestrel.
- c Third generation: desogestrel, gestodene, norgestimate.
- d Fourth generation: drospirenone, dienogest, nomegestrol.
- e Data available for 714 cases and 429 controls.
- f Only first-degree relatives were considered.
- g Data available for 758 cases and 450 controls.
- h At time of VT episode for cases, at time of inclusion for controls.
- i Protein C, protein S and antithrombin deficiencies, homozygous for factor V Leiden, homozygous for G20210A prothrombin mutation, or combined defects.

Genotype and allele distributions of the nine studied SNPs in cases and controls were provided in Table 2. All SNPs were in Hardy–Weinberg equilibrium. Only the *F11* rs2289252

showed statistical significant association with VT risk. The rs2289252-A allele was significantly more frequent in cases than in controls (49% vs 42%) and associated with an adjusted 1.60-fold (1.33–1.94) ($p < 10^{-4}$) increased risk of VT. The magnitude of its effect was similar to that reported in the general population (OR = 1.35) 11. The OR for VT associated with the rs2289252-A allele was similar in smoker and non-smoker women (OR = 1.62 vs OR = 1.63; $p = 0.96$) and in O and non-O carrier women (OR = 1.80 vs OR = 1.50; $p = 0.45$). In addition, the allele frequency of the rs2289252-A allele was similar according to the presence or absence of family history of VT ($p > 0.37$, regardless of the definition used: the existence of a first-degree family history of VT or a family history score above 0.3) 9.

Table 2. Association of studied SNPs with the risk of venous thrombosis in combined oral contraceptive users

	Controls	Cases	pa	ORb	p
rs4524 (<i>F5</i>)	AA 272 (59%)	462 (60%)		1	
	AG 166 (36%)	265 (35%)			
	GG 26 (5%)	39 (5%)		0.90 (0.73–1.12)	0.36
RAFc	0.23	0.22	$p = 0.528$		
rs2289252 (<i>F11</i>)	GG 166 (36%)	195 (25%)		1	
	AG 231 (46%)	387 (51%)			
	AA 85 (18%)	184 (24%)		1.60 (1.33–1.94)	$<10^{-4}$
RAF	0.42	0.49	$p = 1.84 \cdot 10^{-4}$		
rs2036914 (<i>F11</i>)	GG 136 (29%)	266 (35%)		1	
	GA 230 (50%)	363 (47%)			
	AA 98 (21%)	137 (18%)		1.24 (1.04–1.49)	0.02
RAF	0.46	0.42	$p = 0.037$		
rs2066865 (<i>FGG</i>)	GG 272 (59%)	425 (56%)		1	
	AG 159 (34%)	298 (39%)			
	AA 33 (7%)	43 (5%)		1.10 (0.89–1.35)	0.38
RAF	0.24	0.25	$p = 0.648$		
rs867186 (<i>PROCR</i>)	AA 361 (78%)	611 (80%)		1	
	AG 98 (21%)	142 (18%)			
	GG 5 (1%)	13 (2%)		0.88 (0.67–1.17)	0.38
RAF	0.12	0.11	$p = 0.612$		
rs3136516 (<i>F2</i>)	AA 117 (25%)	193 (25%)		1	
	AG 219 (47%)	392 (51%)			
	GG 128 (28%)	181 (24%)		0.93 (0.78–1.11)	0.43
RAF	0.51	0.49	$p = 0.345$		
rs710446 (<i>KNG1</i>)	AA 153 (33%)	215 (28%)		1	
	AG 227 (49%)	393 (51%)			
	GG 84 (18%)	158 (21%)		1.19 (0.99–1.43)	0.06
RAF	0.43	0.46	$p = 0.070$		
rs2288904 (<i>SLC44A2</i>)	AA 18 (4%)	25 (3%)		1	
	CG 160 (35%)	243 (32%)		1.23 (0.98–1.54)	0.08

	Controls	Cases	pa	ORb	p
	GG 285 (62%)	495 (65%)			
RAF	0.79	0.81	p = 0.231		
	CC 1 (<1%)	0 (0%)		1	
rs78707713 (<i>TSPAN15</i>)	CT 84 (18%)	104 (14%)		1.40 (0.99–1.97)	0.06
	TT 378 (82%)	658 (86%)			
RAF	0.91	0.93	p = 0.022		

- CI, confidence interval; COC, combined oral contraceptives; OR, odds ratio; SNP, single nucleotide polymorphisms.
- a Cochran Armitage Trend test p value.
- b Allelic odds ratio (95% CI) adjusted for age, type of COC, duration of COC use and family history score calculated by logistic regression analysis.
- c Allele frequency of the reported risk allele.

As a consequence of these results, women carrying both the *F11* rs2289252-A allele and non-O blood group, representing about 52% of the women population, presented a 4.00-fold (2.49–6.47) ($p < 10^{-4}$) increased risk compared with women of O blood group and rs2289252-GG genotype. We further stratified this analysis according to obesity, one of the main environmental risk factor for VT in COC users, to better assess the risk associated with those two common genetic risk factors for VT. Because of the small number of patients in the highest class of obesity, we merged the top two classes: 30–35 kg/m² and >35 kg/m². Adjusted OR estimating the VT risk associated with different combinations of these three risk factors, rs2289252, ABO blood group and obesity, are presented in Table 3. Women with BMI over 30 kg/m² (class I obese and above) of non-O blood group that harbored the rs2289252-A allele were at 13.15-fold (5.20–38.50) ($p < 10^{-4}$) increased risk compared with non-obese women with O blood group and rs2289252-GG genotype, the former group representing 4.8% of the female population.

Table 3. Impact on VT risk of obesity, ABO blood group and *F11* rs2289252

BMI (kg/m ²)	ABO blood group	<i>F11</i> rs2289252a	Cases, n (%)	Controls, n (%)	OR (95% CI)b	p
<25	O	GG	39 (5.5)	46 (10.9)	1	
25–30	O	GG	4 (0.6)	6 (1.4)	0.99 (0.22–4.03)	0.98
>30	O	GG	0	1 (0.2)	NA	NA
<25	Non-O	GG	89 (12.6)	81 (19.2)	1.51 (0.86–2.63)	0.15
25–30	Non-O	GG	26 (3.7)	15 (3.6)	2.39 (1.07–5.52)	0.04
>30	Non-O	GG	24 (3.4)	5 (1.2)	6.10 (2.17–20.31)	0.01
<25	O	AG/AA	83 (11.7)	77 (18.3)	1.46 (0.83–2.57)	0.18
25–30	O	AG/AA	22 (3.1)	13 (3.1)	3.55 (1.49–8.71)	0.005

BMI (kg/m ²)	ABO blood group	<i>F11</i> rs2289252a	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR (95% CI) ^b	P
>30	O	AG/AA	18 (2.5)	6 (1.4)	5.97 (2.08–19.39)	0.002
<25	Non-O	AG/AA	288 (40.6)	148 (35.2)	2.99 (1.81–4.96)	<10 ⁻⁴
25–30	Non-O	AG/AA	68 (9.6)	17 (4.0)	5.58 (2.75–11.76)	<10 ⁻⁴
>30	Non-O	AG/AA	48 (6.7)	6 (1.4)	13.15 (5.20–38.50)	<10 ⁻⁴

- BMI, body mass index; CI, confidence interval; COC, combined oral contraceptives; NA, not applicable; OR, odds ratio; VT, venous thrombosis.
- a Genotype for *F11* rs2289252. Risk allele = A.
- b Adjusted OR. Adjustment factors = age, type and duration of COC use, and family history score.

Discussion

Efforts to promote a better identification of women at high-risk of COC-related VT are invaluable, as they may lead to more adequate prescription of contraception, with less or no thrombogenic influence and, perhaps, a decrease of these preventable VT events.

In this study, we found that the *F11* rs2289252 is a mild and common genetic risk factor for VT in COC users. The allelic OR associated with the polymorphism was 1.60. The risk allele was common in the population, 64% in the control group, and its impact on VT risk was independent on that of the non-O blood group, another common genetic risk factor. As a consequence, the combination of these two genetic factors that occurred together in the general population at a prevalence of ~52% was associated with an ~fourfold increased risk for VT.

Eight other established VT-susceptibility alleles were investigated in our study, but failed to reach statistical significance. A nominal association ($p = 0.02$) was observed for the rs78707713 whose rare allele tend to associate with increased risk of disease as initially reported 11. The lack of statistical significance was probably because of some power issues, as our sample size was not large enough to detect genetic effects characterized by moderate impact on VT risk. As an illustration, the power of the PILGRIM study to detect allele ORs as low as 1.4 was only 47% for a polymorphism with a minor allele frequency of 0.10 (which corresponds to the observed association at *TSPAN15* rs78707713) at the statistical threshold of 6×10^{-3} . However, we cannot exclude that the genetic associations reported in the general population 10 do not hold in specific groups of subjects such as COC users.

This study underlined the role of combination of common genetic risk factors on the risk of VT in COC users and confirmed the complexity of the disease arising from the occurrence of multiple risk factors. Family history, besides being a recognized risk factor for VT, is poorly associated with known genetic risk factors and thus cannot be considered as a surrogate for the search of these genetic factors. Thrombophilia screening is not recommended in most of the international recommendations in part because defects are rare in the general population 12. Conversely, the combination of the non-O blood group with the rs2289252-A allele is far

from being rare as it accounts for 52% of COC users. Moreover, in our cohort, the magnitude of risk provided by this combination of at-risk allele is similar to that of class II obesity (BMI higher than 35 kg/m²), which is a relative contraindication for COC use in some international recommendations. Very interestingly, the combination of one demographic (partly genetic and partly environmental) risk factor (obesity) and two common genetic risk factors (non-O blood groups and *F11* rs2289252) represented a major risk factor for VT. All current guidelines and recommendations consider the risk associated with COC in women harboring a mild thrombophilia unacceptable. As a consequence, COC use is contraindicated in all women with mild thrombophilia. However, van Vlijmen et al. evaluated in a recent review the additional effect of inherited thrombophilia in COC users and suggested that COC could be prescribed in women with mild thrombophilia in the absence of other risk factors 8. Indeed, most women using COC and harboring mild thrombophilia would not ever undergo VT. As a consequence, environmental and genetic modifiers that may increase the risk of VT in these women need to be identified. In this goal, other common genetic risk factors, such as ABO blood group and *F11* rs2289252, could be taken into account in the VT risk assessment. More generally, as VT is a complex disease, combination of risk factors could be more useful to consider for assessing the risk than to consider each risk factor individually. In this study, the OR associated with the combination of three common risk factors was found to be 13.15 (5.20–38.50), which is higher than the risk ratio (RR) associated with factor V Leiden or prothrombin mutation in COC users according to van Vlijmen et al. meta-analysis (RR = 5.89; 95% CI: 4.21–8.23) 8. Of note, only severe obesity (BMI > 35 kg/m²) might be considered as a contraindication for COC use, whereas a 30 kg/m² cut-off showed a high level of risk in the present study. The magnitude of this effect was highly influenced by the combination based on the two common genetic risk factors, which suggests that this combination could provide useful information for the VT risk assessment in COC users exposed to environmental risk factors such as obesity. The addition of those three risk factors could result in a state of hypercoagulability. Indeed, *F11* rs2289252 is associated with increased plasmatic levels of FXI, which is a key enzyme of the coagulation pathway as it activates factor IX 13. Moreover, thrombin generation is increased in patients with elevated FXI 14. Similarly non-O blood groups are associated with a 30% increased level of factor VIII, which elevation has been shown to increase thrombin generation 15. Finally, obesity is also associated with increased thrombin generation 16. Altogether elevated FXI and FVIII and obesity could increase thrombin generation and VT risk. It is currently unclear whether obesity should contraindicate COC use or not. Obese women (i.e. BMI > 30 kg/m²) could be eligible for the screening of the two common genetic risk factors. Based on their prevalence in general population (non-O blood groups ≈ 50%; *F11* rs2289252 ≈ 60%) the screening would identify the combination of the two alleles in 30% of obese women whom should be considered at high risk.

This study suffers from limitations which can prevent us from generalizing the results. The first one is relative to the study design which can cause bias. The control group is not derived from the general population. Indeed, controls were recruited as they had a family history of thrombosis and thus were putatively enriched in genetic risk factors. This explains why factor V Leiden was not associated with VT while it is the most robust and strongest common genetic risk factor of VT 9. However, despite this putative genetically enriched control group, the study has been able to show a 60% increased risk of VT in women using COC who harbored the risk allele for *F11* rs2289252. Another limitation is that we used a subcohort of PILGRIM (i.e. patients with DNA available). We have checked for homogeneity between the total cohort and the subcohort. Main characteristics of patients were similar between the two groups as well as the impact of the major risk factors for VT. The former point allows us to

consider that this subcohort is representative for the whole PILGRIM cohort. Finally, this study was monocentric and has included individuals only from Marseilles area. Results should thus be replicated in individuals from other countries in order to verify their generalizability.

This study provides evidence of the difficulty in assessing individual risk of VT, which complexity arises from the multiplicity of risk factors that need to be taken into account. In that matter different predictive scores including genetic and environmental risk factors have emerged in the recent literature as useful predictors of VT of an individual 17-19. It is mandatory to validate these scores and design new ones specific for COC users. Effectiveness of these scores should be assessed and compared with current practice in management studies. Their cost-effectiveness in contraceptive counseling should also be evaluated before implementation in clinical practice.

uated before implementation in clinical practice.

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