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The joint effect of genetic risk factors and different types of combined oral contraceptives on venous thrombosis risk

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Abstract

It is not known whether the synergistic effect of genetic markers, increasing the risk of venous thrombosis (VT), and combined oral contraceptives (COC) use varies between different types of progestogens in these preparations. We investigated the joint effect of genetic risk factor, that is, F5 rs6025, F2 rs1799963, and FGG rs2066865 mutations, and different progestogens on the risk of VT. The constrained maximum likelihood estimation (CMLE) method was used to calculate joint effects, expressed as odds ratio (OR) with 95% confidence intervals [CI]. As the dose of estrogen is known to be a risk factor for VT, analyses were restricted to COC with 30 µg estrogen and each progestogen. Overall, the joint effect of COC and genetic variants was lowest for COC containing the progestogen levonorgestrel, albeit CIs were wide. The OR (95% CI) of the four different analyses (i.e. joint effect with F5 rs6025, F2 rs1799963, F5 rs6025 or F2 rs1799963 and FGG rs2066865) ranged between 7·4 (5·4–10·2) and 24·8 (12·3–50·0) for levonorgestrel. For gestodene the joint effect ranged between 11·7 (7·2–19·1) and 30·9 (10·6–89·9). Desogestrel and cyproterone acetate had the highest risk estimates: 14·6 (9·7–21·9) and 32·6 (13·2–80·6) and 15·5 (9·7–24·9) and 44·4 (16·9–116·3) respectively. In women with inherited thrombophilia, COC containing levonorgestrel were associated with the lowest risk of VT, albeit the CIs were wide.

Keywords: contraceptives, oral, combined, thrombosis, progestins, genes, risk.

The risk of venous thrombosis (VT), (i.e. that is, deep vein thrombosis or pulmonary embolism) is increased in women who use combined oral contraceptives (COC).[1–3] Initial attempts to lower the risk were made by reducing the estrogen (ethinylestradiol; [EE]) dose in the COC, which indeed led to a reduced risk of VT.[4–6] Apart from lowering the dose of estrogen, the progestogen component was changed over time to reduce disease frequency, but this was not successful. In contrast, the risk of VT was even higher in users of the more recently developed progestogens. For example, the progestogens desogestrel and drospirenone correspond with a higher VT risk when compared with levonorgestrel.[7–9]

Venous thrombosis is a multifactorial disease[10] caused by environmental factors such as the use of COC, and by genetic risk factors, for example the factor V Leiden (F5 rs6025) or prothrombin (F2 rs1799963) mutations.[11–13] Moreover, COC use and the F5 rs6025 mutation have a synergistic effect (SI) on VT, with a 30-fold increased risk of VT in COC users carrying this mutation, compared with women who are not COC users and non-carriers of F5 rs6025.[1] Additional to the F5 rs6025 and F2 rs1799963 mutations, another genetic variant which is consistently associated with VT (literature average OR for VT: 1·56)[14] and has a high prevalence (6%) in the general population, is fibrinogen gamma (FGG rs2066865).[15] This variant leads to reduced levels of an alternatively spliced variant of fibrinogen-ɣ, called 'ɣ' , and ultimately to more thrombus formation and stabilization.[16,17] A case–control study showed that the FGG mutation increased the risk of VT with an OR of 2·01 (95% CI: 1·23–3·31).[18] There is no information available on the SI between COC use and the FGG rs2066865 variant on the risk of VT.

Currently, it is known that the risk of VT varies between different types of progestogens in COC; however, it is still

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not known whether the SI with genetic variants associated with VT risk, varies for the different progestogens in the COC.

The aim of this study was to investigate the joint effect between a genetic risk factor for VT, (i.e. F5 rs6025, F2 rs1799963 and FGG rs2066865 mutations) and the use of COC on the risk of VT. The joint effect was assessed for COC use in general and for the different progestogens in COC separately.

Methods

Study design and study participants

For the analysis, data from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study were used.[19] The MEGA study is a large, population-based, case–control study conducted between 1999–2004. In this study, patients with a first episode of deep vein thrombosis (DVT) or pulmonary embolism (PE) were included from the records of six participating anticoagulant clinics in the Netherlands. Information on the diagnostic procedure was obtained from hospital records and general practitioners. DVT was confirmed with Doppler ultrasonography and PE was confirmed using a ventilation perfusion lung scan, spiral computed tomography or angiogram. The controls used in this study were either the partners of patients or recruited by random digit dialling (RDD). The random controls were frequency matched to the patients with respect to age and sex. Individuals with severe psychiatric problems or those unable to speak Dutch were considered ineligible.

For the current analyses, we selected female participants aged 18–49 years. Exclusion criteria were women who were postmenopausal, pregnant, or within 4 weeks postpartum at the time of thrombotic event or index date (see below) and women using hormonal contraception methods other than COC. We analysed frequently used COC containing EE combined with the following progestogens: gestodene, desogestrel, levonorgestrel and cyproterone acetate. Because of the low numbers in cases and controls, analysis could not be performed for COC containing other types of progestogens. For the analyses, we pooled the two control groups into a single group. For the final analyses, we included 1426 cases and 1777 controls (716 partners and 1061 RDD controls).

Data collection and laboratory analysis

All women filled in a standardized questionnaire on risk factors of VT, such as family history of thrombosis, pregnancy and the use of oral contraceptives in the year before the index date.

A positive family history was defined as a participant having at least one parent or sibling with a history of VT.

The index date was the date of the thrombotic event for patients and their partners and the date of filling in the questionnaire for the random controls. The questionnaire was sent to all patients and their partners within a few weeks after the index date. For the random controls, the questionnaire was sent after they agreed to participate.

At least 3 months after discontinuation of the oral anticoagulation therapy, patients and their partners were invited to the anticoagulation clinic for an interview and blood sample or buccal swab for DNA extraction. After completing the questionnaire, RDD controls were also invited for an interview and blood sample. Details on the current use of oral contraceptives were verified during the interviews. The genetic risk factors F5 rs6025, F2 rs1799963 and FGG rs2066865 were determined by polymerase chain reaction with use of the TaqMan assay. Further details of DNA extraction and analysis are described elsewhere.[20]

Statistical analysis

Odds ratios were calculated to quantify the effect of COC use alone, the genetic risk factor(s) alone, and the joint effect of COC and the genetic risk factor(s), all compared with non-users and non-carriers. Furthermore, the SI, to quantify joint effect of COC and the genetic risk factor(s), was calculated. The SI (multiplicative scale) is a measure to quantify interaction between genes and environmental factors,[21] which reflects the additional effect of the gene and COC combination above the effect of the gene alone or the COC use alone. As the number of control participants carrying one of the genetic variants and using (a specific type of) COC was small, standard logistic regression will give an imprecise estimate of the joint effect [wide confidence interval (CI)]. Under the assumption of independence between genes and the prescription of COC, the coefficients of the logistic regression model can be estimated more precisely by using Constrained Maximum Likelihood Estimation (CMLE).[22] The method is available in the R package CGEN: https://bioconductor.org/packages/release/bioc/html/CGEN.html.

As mentioned above, an important assumption of the CMLE method is that independence between the genetic and environmental exposure in the population, that is, that the prescription of COC (and the different progestogen types in COC) does not differ between carriers of the genetic risk factor(s) and non-carriers. A previous study used doctor's preference for a certain COC type as an instrumental variable to assess the risk of VT associated with third versus second generation oral contraceptives and found similar risk estimates compared with conventional analysis.[23] This suggests that the doctor's preference for a certain COC type is a valid instrumental variable and that prescription bias is unlikely.

However, to verify the assumption, in the controls we compared the distribution of the different progestogen types in COC in women with and without a family history of VT. Furthermore, we performed a sensitivity analysis in the

subgroup of women without family history. We also assessed the distribution of the different progestogen types in COC in controls with and without the genetic risk factor(s).

Using the CMLE method, we reported OR with 95% CI for the joint effects between F5 rs6025, F2 rs1799963 and FGG rs2066865 and COC in general. Subsequently, we performed the same analysis, stratified for the different progestogen types in COC. Since the dose of estrogen is known to be a risk factor for VT, we performed this analysis restricted to COC with 30 μg EE (the most common type of COC used in the MEGA study) and each progestogen type in COC. The COC containing cyproterone acetate (Diane-35) always contained 35 μg of EE, as this is the only available concentration.

Since F5 rs6025 and F2 rs1799963 are often reported together in the literature and in order to increase the power of our study, we also performed the analysis in women carrying either one of these two genetic risk factors. All analyses were adjusted for age.

As a sensitivity analysis we performed standard logistic regression. As a comparison, the results are reported in the Tables SIV–SVII. All analyses in this study were performed with R package i368 version 3.6.0.

Results

Baseline characteristics are shown in Table I. In total, 1426 cases and 1777 controls were analysed. Both cases and controls using COC were younger, with a mean age (range) of 36 years (18–50 years) for cases and 34 years (18–50 years) for controls compared with cases and controls who were non-users, 41 (19–50) and 40 years (18–50 years) respectively.

There was no difference in the distribution of progestogen types in COC in controls with and without family history and there was a slight difference in the distribution of the progestogen types in women with and without genetic risk factor(s). However, this was not in the expected direction since for the large part, a lower proportion of women with a genetic risk factor(s) were prescribed a COC containing levonorgestrel, which was reported to be the safest preparation.

Table II shows the individual effects of F5 rs6025, F2 rs1799963, F5 rs6025 or F2 rs1799963 and FGG rs2066865 and COC in general, the SI and the joint effect between these genetic factor(s) and COC in general. The genetic factors as well as oral contraceptive use increased the risk of VT. However, the risk was highest when both risk factors were present.

The joint effect of genetic risk factor(s) and COC in general was OR (95% CI) 19·3 (13·9–26·8) for F5 rs6025 and COC; 24·0 (13·6–42·3) for F2 rs1799963; 20·5 (15·0–27·8) for F5 rs6025 or F2 rs1799963; and 8·9 (6·9–11·5) for FGG rs2066865.

Table III show results stratified for the different progestogen types and restricted to 30 μg of EE in COC. The OR (95% CI) of the four different analysis (i.e. joint effect with F5 rs6025, F2 rs1799963, F5 rs6025 or F2 rs1799963 and FGG rs2066865) ranged between 7·4 (5·4–10·2) and 24·8 (12·3–50·0) for levonorgestrel. For gestodene the joint effect ranged between 11·7 (7·2–19·1) and 30·9 (10·6–89·9). The progestogens desogestrel and cyproterone acetate had the highest risk estimates for the joint effects, that is, 14·6 (9·7–21·9) and 32·6 (13·2–80·6) and 15·5 (9·7–24·9) and 44·4 (16·9–116·3) respectively.

The SI was increased for all combined effects indicating a multiplicative interaction for all types of COC. Nonetheless, overall, the joint effect of COC and a genetic variant compared with non-users and non-carriers, was lowest for COC containing the progestogen levonorgestrel, although the CIs were wide.

In Table IV, we further analysed the joint effects between genetic factors and COCs containing gestodene, desogestrel, or cyproterone acetate versus those containing levonorgestrel. Overall, the risk for the joint effect was higher for the progestogens gestodene, desogestrel and cyproterone acetate compared with levonorgestrel, albeit the CIs of all the risk estimates were wide.

Similar results were obtained in the sensitivity analysis in women without a family history of VT (Tables SI–SIII). Furthermore, similar results were obtained by the standard logistic regression analysis (Tables SIV–SVII).

Discussion

We assessed the risk of VT for the joint effects of COC and genetic variants using the CMLE method. We have shown that in women with inherited thrombophilia, COC use further increased the risk of VT. When restricting the analysis to COC with 30 μg of EE, the joint effect of genetic risk factor(s) and COC containing the progestogen levonorgestrel was associated with the lowest risk of VT, albeit the CIs were wide.

When comparing the relative risks of the joint effects between the different genetic factors and COC use in general, we observed that all joint effects were lower for FGG rs2066865 compared with F5 rs6025 and F5 rs6025 or F2 rs1799963 combined. This is to be expected since FGG rs2066865 is more prevalent in the population, but is associated with a lower relative risk of VT compared with the two other genetic variants.[11]

A recent systematic review and meta-analysis showed that the presence of mild F5 rs6025 or F2 rs1799963 mutation and severe thrombophilia (antithrombin, protein C or protein S deficiency, and double heterozygosity and homozygosity of F5 rs6025 or F2 rs1799963 mutation) increases the risk of VT in COC users 6-fold and 7-fold respectively.[24] Other studies have shown that the absolute risks are 0·49–2·0 per 100 pill-years for mild and 4·3–4·6 per 100 pill-years for severe thrombophilia.[25–27] The incidence of VT in COC users with double heterozygosity or homozygosity of F5 rs6025 or

Table I. Baseline characteristics of study participants.

	Non-users	Contraceptive users	Gestodene	Desogestrel	Levonorgestrel	Cyproterone
Cases, n = 1426*	349	1073	115	267	452	115
Mean age in years (range)	41 (19–50)	36 (18–50)	36 (20–49)	35 (18–50)	37 (18–50)	32 (18–50)
Without family history, n (%)	212/289 (73·4)	703/920 (76·4)	75/100 (75)	174/226 (77·0)	296/393 (75·3)	80/98 (81·6)
With family history†, n (%)	77/289 (26·6)	217/920 (23·6)	25/100 (25)	52/226 (23·0)	97/393 (24·7)	18/98 (18·4)
Without F5	266/294 (90·5)	800/976 (82·0)	87/104 (83·7)	209/248 (84·3)	328/410 (80·0)	83/104 (79·8)
With F5	28/294 (9·5)	176/976 (18·0)	17/104 (16·3)	39/248 (15·7)	82/410 (20·0)	21/104 (20·2)
Without F2	275/294 (93·5)	925/976 (94·8)	99/104 (95·2)	237/248 (95·6)	384/410 (93·7)	97/104 (93·3)
With F2	19/294 (6·5)	51/976 (5·2)	5/104 (4·8)	11/248 (4·4)	26/410 (6·3)	7/104 (6·7)
Without F5 or F2	247/294 (84·0)	759/976 (77·8)	82/104 (78·8)	200/248 (80·6)	307/410 (74·9)	79/104 (76·0)
With F5 or F2	47/294 (16·0)	217/976 (22·2)	22/104 (21·2)	48/248 (19·4)	103/410 (25·1)	25/104 (24·0)
Without FGG	130/295 (44·1)	430/976 (44·1)	45/104 (43·3	120/248 (48·4)	177/410 (43·2)	37/104 (35·6)
With FGG	165/295 (55·9)	546/976 (55·9)	59/104 (56·7)	128/248 (51·6)	233/410 (56·8)	67/104 (64·4)
Controls, n= 1777*	1071	699	67	105	353	61
Mean age in years (range)	40 (18–50)	34 (18–50)	35 (19–49)	34 (20–50)	34 (18–50)	31 (19–49)
Without family history, n (%)	771/897 (86·0)	519/578 (89·8)	54/58 (93·1)	78/87 (89·7)	267/298 (89·6)	43/49 (87·8)
With family history†, n (%)	126/897 (14·0)	59/578 (10·2)	4/58 (6·9)	9/87 (10·3)	31/298 (10·4)	6/49 (12·2)
Without F5	745/797 (93·5)	463/487 (95·1)	51/55 (92·7)	70/75 (93·3)	236/247 (95·5)	38/38 (100)
With F5	52/797 (6·5)	24/487 (4·9)	4/55 (7·3)	5/75 (6·7)	11/247 (4·5)	0/38 (0)
Without F2	785/797 (98·5)	481/487 (98·8)	53/55 (96·4)	75/75 (100)	244/247 (98·8)	38/38 (100)
With F2	12/797 (1·5)	6/487 (1·2)	2/55 (3·6)	0/75 (0)	3/247 (1·2)	0/38 (0)
Without F5 or F2	733/797 (92·0)	457/487 (93·8)	49/55 (89·1)	70/75 (93·3)	233/247 (94·3)	38/38 (100)
With F5 or F2	64/797 (8·0)	30/487 (6·2)	6/55 (10·9)	5/75 (6·7)	14/247 (5·7)	0/38 (0)
Without FGG	432/802 (53·9)	258/488 (52·9)	25/55 (45·5)	43/75 (57·3)	129/248 (52·0)	24/38 (63·2)
With FGG	370/802 (46·1)	230/488 (47·1)	30/55 (54·5)	32/75 (42·7)	119/248 (48·0%)	14/38 (36·8%)

F5, Factor V Leiden rs6025; F2, (prothrombin) F2 rs1799963; FGG, fibrinogen gamma rs2066865.

*Type of contraceptive used is missing for 16 cases and 19 controls. Of 4 cases and 7 controls it was not known whether they were users or non-users.

†With family history is defined as having at least one parent or sibling with a history venous thrombosis.

F2 rs1799963 mutation was 0·86 per 100 pill-years, suggesting that the absolute risk of this double defect is less serious than an antithrombin, protein C or protein S deficiency.[27] In most case–control studies included in the meta-analysis, the number of thrombophilic controls who used COC was small, yielding unreliable odds ratios of VT. One of the case–control studies with sufficient power suggested that the risk of VT associated with the joint effect of F5 rs6025 and oral contraceptive use is increased 15-fold compared with wildtype carriers without oral contraceptive use, rather than the previously reported 30-fold increased risk.[28] Currently, the World Health Organization (WHO) states that COC use in women with inherited thrombophilias is associated with an unacceptably high risk of VT.[29] The authors of the meta-analysis suggested that COC could be prescribed in women with mild thrombophilia (without family history of VT), when alternative forms of contraception are not well tolerated. Although COC can be considered in women with certain genetic risk factors, we have shown in our study that, most likely, the type of COC prescribed matters. If COC use is considered in this high risk group, similar to the general population, the COC containing the progestogen levonorgestrel is preferable as it was associated with the lowest risk of VT. The reference group in our study, which was used to compare the risks between different types of progestogens, was similar and, therefore, a direct comparison between the progestogens was possible. Nevertheless, as the CIs are wide and absolute risks are lacking, interpretation of these results should be done with caution and larger studies are needed to confirm these findings.

A recent study[30] assessed the SI between F5 rs6025 and the different generations of COC. They have shown that the SI for the joint effect with F5 rs6025 was the lowest for the first/second generation COC (containing the progestogens norethisterone, lynestrenol and levonorgestrel) compared with third (gestodene and desogestrel) generation COC, cyproterone acetate and drospirenone. In contrast, our SI for the most part was higher for the progestogen levonorgestrel compared with the other three progestogens, gestodene, desogestrel and cyproterone acetate, although the CIs of the SIs also overlap (Table III). However, the aim of this study was not to compare the SI, but the joint effect, which also takes into account the individual effects (risk). The SI is not a clinically relevant measure. The SI is a statistical measure which is needed to calculate the joint effect and may not be appropriate as the main outcome of a study. If the OR of COC only is high, then the combined effect will also be high while the SI may be low (or the other way

Table II. Individual effects, joint effect and synergy index of genetic risk factors and combined oral contraceptives in general on venous thrombosis risk.

		Cases	Controls	OR (95% CI)†	SI*
F5	COC				
−	−	266	745	1	
+	−	28	52	1·7 (1·1–2·6)	
−	+	800	463	5·5 (4·6–6·7)	
+	+	176	24	19·3 (13·9–26·8)‡	2·1 (1·4–3·2)
F2	COC				
−	−	275	785	1	
+	−	19	12	4·9 (2·5–9·4)	
−	+	925	481	6·2 (5·1–7·5)	
+	+	51	6	24·0 (13·6–42·3)‡	0·8 (0·5–1·4)
F5 or F2	COC				
−	−	247	733	1	
+	−	47	64	2·4 (1·7–3·5)	
−	+	759	457	5·7 (4·7–6·9)	
+	+	217	30	20·5 (15·0–27·8)‡	1·5 (1·1.-2·1)
FGG	COC				
−	−	130	432	1	
+	−	165	370	1·5 (1·1–1·9)	
−	+	430	258	6·1 (4·8–7·7)	
+	+	546	230	8·9 (6·9–11·5)‡	1·0 (0·8–1·3)

F5, Factor V Leiden rs6025; F2, (prothrombin) F2 rs1799963; FGG, fibrinogen gamma rs2066865; COC, combined oral contraceptives; OR, odds ratio; CI, confidence interval; SI, synergy index.

*Synergy index calculated using the constrained maximum likelihood (CMLE) method.

†Adjusted for age.

‡Joint effect calculated using the CMLE method.

around). The joint effect is, therefore, much more interesting from a clinical point of view regarding the safety of the different COC preparations in the women with a genetic mutation. Indeed, the joint effect in our study was the lowest for the progestogen levonorgestrel, although the CIs were wide.

Hugon-Rodin et al. grouped the different progestogens into generations. As several studies have shown that the risk of VT varies for the individual progestogens in COC, which are often grouped together into generations. For example, the progestogens desogestrel and gestodene are grouped as third generation COC, but desogestrel is associated with a higher risk of VT compared with gestodene.[7] We therefore assessed the joint effects for each type of progestogen in COC and genetic risk factors separately. We also included the joint effects for two other important and prevalent genetic risk factors, that is, F2 rs1799963 and FGG rs2066865, rather than F5 rs6025 alone.

Apart from the strengths that are mentioned above, other strengths of this study are that it is a large, population-based, case–control study with objectively confirmed VT. Also, assessment of a large number of genetic and environmental risk factors of VT was done enabling us to assess the joint effects between genetic factors and oral contraceptives containing different types of progestogens.

Limitations are that, even though this is a very large study, there was only a small number of controls with genetic risk factors after stratification for COC containing different types of progestogens. However, this limitation was overcome to some extent by using the CMLE method to estimate ORs, which gives more precise estimates and smaller CIs compared with the traditional case–control approach, when one can assume that there is independence between genetic and environmental risk factors. Nonetheless, even with the CMLE method our CIs were wide.

Usually, both the doctor and the woman requesting COC are unaware of a genetic predisposition for VT at the time of COC prescription. However, a positive family history of VT could be seen as a proxy for the presence of a genetic predisposition for VT. A doctor may preferentially prescribe certain types of COC to women with or without a positive family history of VT. However, a previous study showed that a positive family history of VT corresponded poorly with known genetic risk indicating that prescription bias is unlikely.[31] Nevertheless, we still assessed the distribution of the different types of progestogens in COC in women with and without family history and found that there is no difference between these two groups. We also performed a sensitivity analysis in women without family history. The results were in a similar direction compared to the results from the main analysis.

Table III. Individual effects, joint effect and synergy index of genetic risk factors and different progestogen types in combined oral contraceptives on VT risk (Restriction analysis).

		Gestodene				Desogestrel				Levonorgestrel				Cyproterone			
		Cases	Controls	OR (95% CI)†	SI*	Cases	Controls	OR (95% CI)†	SI*	Cases	Controls	OR (95% CI)†	SI*	Cases	Controls	OR (95% CI)†	SI*
F5	COC																
–	–	266	745	1		266	745	1		266	745	1		266	745	1	
+	–	28	52	1·6 (1·0–2·6)		28	52	1·6 (1·0–2·6)		28	52	1·6 (1·0–2·6)		28	52	1·6 (1·0–2·6)	
–	+	74	34	7·0 (4·5–10·7)		168	52	10·5 (7·4–14·8)		216	164	4·3 (3·3–5·6)		83	38	8·0 (5·2–12·3)	
+	+	15	2	22·1 (11·3–43·3)‡	1·9 (1·0–3·8)	27	4	26·3 (15·2–45·5)‡	1·5 (0·9–2·7)	56	8	17·4 (11·4–26·6)‡	2·5 (1·5–4·0)	21	0	31·8 (17·2–59·0)‡	2·4 (1·3–4·5)
F2	COC																
–	–	275	785	1		275	785	1		275	785	1		275	785	1	
+	–	19	12	4·7 (2·4–9·2)		19	12	4·7 (2·4–9·2)		19	12	4·7 (2·4–9·2)		19	12	4·7 (2·4–9·2)	
–	+	84	35	7·7 (5·0–11·7)		187	56	11·3 (8·0–15·8)		253	169	4·9 (3·8–6·3)		97	38	9·1 (6·0–13·8)	
+	+	5	1	30·9 (10·6–89·9)‡	0·9 (0·3–2·4)	8	0	32·6 (13·2–80·6)‡	0·6 (0·3–1·4)	19	3	24·8 (12·3–50·0)‡	1·1 (0·6–2·1)	7	0	44·4 (16·9–116·3)‡	1·0 (0·4–2·6)
F5 or F2	COC																
–	–	247	733	1		247	733	1		247	733	1		247	733	1	
+	–	47	64	2·4 (1·6–3·5)		47	64	2·4 (1·6–3·5)		47	64	2·4 (1·6–3·5)		47	64	2·4 (1·6–3·5)	
–	+	69	33	7·0 (4·5–10·8)		161	52	10·8 (7·6–15·3)		201	161	4·3 (3·3–5·6)		79	38	8·2 (5·4–12·7)	
+	+	20	3	25·2 (13·6–46·5)‡	1·5 (0·8–2·7)	34	4	28·3 (17·0–47·1)‡	1·1 (0·7–1·8)	71	11	18·9 (12·8–27·9)‡	1·9 (1·2–2·8)	25	0	32·3 (18·0–58·0)‡	1·7 (1·0–2·9)
FGG	COC																
–	–	130	432	1		130	432	1		130	432	1		130	432	1	
+	–	165	370	1·5 (1·1–1·9)		165	370	1·5 (1·1–1·9)		165	370	1·5 (1·1–1·9)		165	370	1·5 (1·1–1·9)	
–	+	37	16	7·2 (4·3–11·9)		97	33	12·4 (8·4–18·3)		115	89	4·7 (3·4–6·4)		37	24	7·4 (4·5–12·2)	
+	+	52	20	11·7 (7·2–19·1)‡	1·1 (0·7–1·8)	98	23	14·6 (9·7–21·9)‡	0·8 (0·6–1·1)	157	84	7·4 (5·4–10·2)‡	1·1 (0·8–1·5)	67	14	15·5 (9·7–24·9)‡	1·4 (0·9–2·3)

This analysis was restricted to combined oral contraceptives (COC) containing 30 μg of ethinylestradiol (EE) and the respective progestogen (gestodene, desogestrel, levonorgestrel and cyproterone acetate [35 μg of EE]).

F5, Factor V Leiden rs6025; F2, (prothrombin) F2 rs1799963; FGG, fibrinogen gamma rs2066865; OR, odds ratio; CI, Confidence Interval; SI, synergy index.

*SI, synergy index calculated using the constrained maximum likelihood (CMLE) method.

†Adjusted for age.

‡Joint effect calculated by using the CMLE method.

Table IV. Comparing joint effects between genetic risk factors and progestogen types in combined oral contraceptives on VT risk.

	F5 OR (95% CI)	F2 OR (95% CI)	F5 or F2 OR (95% CI)	FGG OR (95% CI)
	Restriction*	Restriction*	Restriction*	Restriction*
Levonorgestrel	1	1	1	1
Gestodene	1.3 (0.6–2.5)	1.2 (0.4–3.6)	1.3 (0.7–2.5)	1.6 (1.0–2.5)
Desogestrel	1.5 (0.9–2.6)	1.3 (0.5–3.2)	1.5 (0.9–2.5)	2.0 (1.3–2.9)
Cyproterone acetate	1.8 (1.0–3.4)	1.8 (0.7–4.6)	1.7 (1.0–3.1)	2.1 (1.3–3.3)

All analysis are adjusted for age.

F5, Factor V Leiden rs6025; F2, (prothrombin) F2 rs1799963; FGG, Fibrinogen Gamma rs2066865; OR, Odds Ratio; CI, Confidence Interval.

*This analysis was restricted to COC containing 30 μg EE and the respective progestogen [gestodene, desogestrel, levonorgestrel and cyproterone acetate (35 μg of EE)].

There was a slight difference in the distribution of the progestogen types in women with and without genetic risk factor(s). However, this was not in the direction that one would expect if there was prescription bias, since a lower proportion of women with a genetic risk factor(s) were prescribed a COC containing levonorgestrel (Table I). This suggests again that prescription bias is unlikely, and the independence assumption likely holds. Furthermore, we were unable to assess the joint effects between other types of oral contraceptives, such as COC containing the progestogen drospirenone and other second generation COC (norgestimate and norethisterone), because of the small number of users of these types of COC in our database.

Another limitation is that besides age, further adjustment of potential confounders was not feasible because of the small sample size.

The risk of VT in women with mild and severe (limited to double homozygosity or heterozygosity for F5 rs6025 or F2 rs1799963) thrombophilia who also use oral contraceptives is modest.

Therefore, screening of asymptomatic women from families with mild and severe thrombophilia is not indicated when starting oral contraceptive.[32] Currently, a positive family history is used as a tool to guide physicians to balance the risk–benefit of using COC. However, we are currently unable to accurately predict who will develop COC related VT. Furthermore, a positive family history was shown to correspond poorly with known genetic risk markers.[31] Knowledge about the presence or not of any important genetic variant could help the physician to balance the risk–benefit when it is necessary to use these drugs, though currently this is not proven yet. Current results indicate that in women who carry the F5 rs6025 or F2 rs1799963 mutation, detailed counselling on all contraceptive choices may be indicated to enable them to make an informed decision on the optimal contraceptive. If they are intolerant to other forms of contraceptives, COC could be offered to these women, as in this situation the increased risk of pregnancy-related VT outweighs the COC-associated risk.[24] However, if a COC is preferred, based on the results of this study, the COC containing the progestogen levonorgestrel with 30 μg of EE was associated with the lowest risk of VT (although with wide CIs). Larger studies, which also report the absolute risks are needed to confirm these findings.

Author contributions

DK, SlC, and AvHV took responsibility for the integrity of the data and the accuracy of the data analysis. AvHV, SlC, and DK, designed the study. DK, and SlC, analysed and interpreted the data. DK and AvHV drafted the manuscript. SlC, WL, SC, FR, critically revised the manuscript for important intellectual content.

Conflicts of interest

The authors declare no conflicts of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Individual effects, joint effect and synergy index of genetic risk factors and combined oral contraceptives in general on VT risk (Without family history of VT).

Table SII. Individual effects, joint effect and synergy index of genetic risk factors and different progestogen types in combined oral contraceptives on VT risk (Restriction analysis and Without family history of VT).

Table SIII. Comparing joint effects between genetic risk factors and progestogen types in combined oral contraceptives on VT risk (Without family history).

Table SIV. Individual effects, joint effect and synergy index of genetic risk factors and combined oral contraceptives in general on VT risk.

Table SV. Individual effects, joint effect and synergy index of genetic risk factors and different progestogen types in combined oral contraceptives on VT risk (Restriction analysis).

Table SVI. Individual effects, joint effect and synergy index of genetic risk factors and combined oral contraceptives in general on VT risk (Without family history of VT).

Table SVII. Individual effects, joint effect and synergy index of genetic risk factors and different progestogen types in combined oral contraceptives on VT risk (Restriction analysis and Without family history of VT).

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