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Risk of thrombosis associated with oral contraceptives in women from 97 families with inherited thrombophilia: high risk of thrombosis in carriers of the G20210A mutation of the prothrombin gene

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Background and Objectives. Oral contraceptives (OC) and inherited thrombophilia are well-known risk factors associated with venous thromboembolism (VTE). However, there are only few studies on the risk of VTE in women with inherited thrombophilia who use oral contraceptives.

Design and Methods. We performed a retrospective family cohort study of 325 women belonging to 97 families with inherited thrombophilia, including antithrombin, protein S and C deficiencies, the factor V Leiden mutation (FVL) and the G20210A mutation of the prothrombin gene (PT20210A) to determine the risk of VTE associated with OC intake.

Results. For carriers of the PT20210A mutation, the risk of VTE in OC users was 3-fold higher (95% CI 1.3-6.8) than that in non-carriers. Carriers of FVL mutation taking OC showed an OR of 1.4 (95% CI 0.6-3.3), indicating a tendency to increased risk of VTE.

Interpretation and Conclusions. Because of the high prevalence of the PT20210A (6.5%) and FVL (2%) mutations in the general Spanish population and the increased risk of VTE associated with OC intake, genetic screening for these mutations should be considered in potential OC users belonging to families with thrombophilia.

Key words: inherited thrombophilia, oral contraceptives, venous thromboembolism.

Oral contraceptives (OC) and inherited thrombophilia (a genetically determined tendency to venous thromboembolism that develops in young patients aged less than 45 years old and tends to be recurrent) are well-known risk factors associated with venous thromboembolism (VTE). Since 1961, when the first association of OC and VTE was reported,[1] many studies have attempted to assess the risk of first VTE for OC users. Most of these studies were case-control, cohort and follow-up studies.[2-8] Selection criteria in the majority of the studies mentioned above included women with VTE who did or did not use OC (cases) compared with controls (women without VTE) and a family history of VTE (venous thrombosis in at least one first or second-degree family member) was considered as another independent risk factor of VTE. There are some case-control studies and some family studies that have investigated the risk of VTE in carriers of antithrombin (AT), protein S (PS) and protein C (PC) deficiencies, the factor V Leiden mutation (FVL) and the G20210A mutation of the prothrombin gene (PT20210A).[5,8-16] All of these showed an increased risk of thrombosis compared with the risk in patients without inherited thrombophilic defects. There are, however, few case-control studies on the risk of VTE in women with inherited thrombophilia who use OC.[3,9,13,15-19] In relation to PC, PS and AT deficiencies, the risk of VTE associated with OC intake was not similar among those studies. Only four family cohort studies have been reported comparing the thrombotic risk in patients with coagulation defects and the role of environmental factors predisposing to VTE.[19-22] Results obtained in these studies regarding the role of OC

intake and VTE are controversial. No family studies have evaluated the risk of VTE in carriers of the PT20210A mutation who use OC. To our knowledge, this is the first family study that includes families with carriers for the PT20120A mutation.

We performed a retrospective family cohort study of 325 women belonging to 97 families with inherited thrombophilia, including AT, PS and PC deficiencies, and the FVL and the PT20210A mutations. Our aims were to determine the risk of VTE associated with OC intake and assess the need to screen the relatives of patients with inherited thrombophilia.

Design and Methods

Selection criteria

From March 1989 to January 1999, 325 women from 97 families were seen at our Unit for thrombophilia screening. Families were included in the study if one or more inherited thrombophilic factors (AT, PC and PS deficiencies, FVL and PT20120A mutations) were identified in at least two family members (including the proband), with or without VTE. The probands were asked to bring as many first and second-degree women relatives as possible to our center. The criterion for the diagnosis of protein deficiency was a plasma level below the lower limit of normal, as used in our laboratory. These lower limits are 80% for AT, 70% for protein C; 73% and 72% for total and free protein S, respectively, in women and men older than 44 years old ; 63% and 54% for total and free protein S in women younger than 45 years old. Information was obtained by questionnaires filled out during an appointment or by a telephone interview. The term VTE was used to describe deep vein thrombosis whether or not the condition was complicated by pulmonary embolism. Standard objective diagnostic procedures were used for all symptomatic women. The age at the time of the thrombosis associated with OC use and the site of thrombosis were recorded. We included women with VTE aged between 15 and 49 years. A carrier of the PT20210 or the FVL mutations was defined as a person heterozygous or homozygous for one of these mutations. Exclusion criteria included pregnancy, puerperium and other hormonal treatments (such as progestagen preparations).

The OC types were classified as follows: first generation OC when containing derivatives of nortestosterone (norethisterone and lynestrenol) and 50 µg of ethynylestradiol; second generation OC when containing levonorgestrel and 30 µg of ethynyloestradiol; and third generation OC when containing desogestrel or gestodene and 30 µg of ethynylestradiol.[5]

Laboratory determinations

Antithrombin and protein C were determined using chromogenic substrates from Chromogenix (Stockholm, Sweden) (normal ranges were from 80% to 110% for AT and from 70% to 130 % for PC). Anticoagulant activity of protein C was measured using reagents from Behring (Marburg, Germany) (normal range from 70% to 150%). Total and free PS were assayed by an enzyme-linked immunosorbent assay (ELISA) method from Diagnostica Stago (normal range from 75% to 140%). Other antigenic measurements were performed only if functional assays were below the normal range [e.g. antigenic antithrombin that was measured by Laurell´s method and antigenic protein C that was measured by an ELISA method (Asserachrom PC, Stago, Asnières, France)]. All of them are performed as standard protocols in our laboratory. DNA was extracted using a standard protocol.[23] We genotyped the PT20210A and the FVL mutations using four previously described primers in a multiplex polymerase chain reaction (PCR), with minor modifications in the reaction conditions. Briefly, 50 µL of mixture containing 20 mM TRIS HCl pH 8.2, 2 mM MgCl, 0.2 mM of each of the four dNTPs, 0.5 µM of each primer, 250 ng of DNA and 1.5 U Taq-gold (Perkin-Elmer) was subjected to 40 cycles of 10 min at 95°C, 1 min at 55°C (for FVL and PT20210A) and 1 min at 72°C with a final extension step of 10 min at 72°C. In the FVL-PT20211A multiplex PCR, the 175 bp and 118 bp PCR products were digested with *TaqI* (Biolabs) and electrophoresed on a 3% Nusieve GTG agarose gel (FMC Bioproducts, Rockland, ME, USA).[24]

Statistical Analysis

The results are expressed as means ± standard deviation for quantitative variables and in percentages with the 95% confidence intervals (CI), for qualitative variables. The differences between qualitative variables were analyzed by using the χ^2-test. A Kaplan-Meier analysis was used to estimate the thrombosis-free survival (in years) of the different groups. The population considered as the reference group was composed of the relatives who were normal for all of the inherited thrombophilic factors. Cox regression analysis was employed to determine the lifetime risk of developing VTE with respect to individuals without defects. We performed conditional logistic regression to estimate

Table 1. Cases of thrombosis in women with or without inherited thrombophilic factors, whether or not using OC and the number of families depending on the type of thrombophilic defect.

	Families	*Cases*	*VTE with OC*	*VTE without OC*	*NO VTE with OC*	*No VTE without OC*
No defect		108 (32.2%)	10	1	31	66
Defects						
PT20210A carriers	41	78 (24%)	12	8	31	27
FVL carriers	12	26 (8%)	6	5	4	11
Protein C	7	34 (10.5%)	2	13	2	17
Protein S	12	30 (9.2%)	1	16	3	10
Antithrombin	2	6 (1.8%)	1	3	–	2
PT20210A+ FVL	6	15 (4.6%)	5	4	3	3
PT20210A+ protein C	7	6 (1.8%)	1	1	1	3
PT20210A+ protein S	6	8 (2.5%)	3	2	1	2
PT20210A+ antithrombin	2	3 (0.9%)	1	–	–	2
FVL +protein C	1	5 (1.5%)	2	3	–	Any case
FVL +protein S	2	4 (1.2%)	1	2	–	1
FVL + antithrombin	–	2 (0.6%)	–	2	–	Any case
Total	97	325	45	60	76	144

VTE: venous thromboembolism. OC: oral contraceptive, PT20210A: carriers of the G20210A mutation of the prothrombin gene mutation, FVL: carriers of the factor V Leiden mutation.

the *odds ratio* (OR) and 95% CI for the different defects. For conditional logistic regression analysis, we generated a variable called *other deficiencies* that included patients with AT, PS and PC deficiencies. *Non-carriers or no-deficiency*, as used in the statistical analysis, were defined as women without the mutation or deficiency analyzed.

Results

The studied population consisted of 325 women belonging to 97 families (12 families with PS deficiency, 7 families with PC deficiency, 2 with AT deficiency, 12 with the FVL mutation, 41 with PT20210A mutation, 1 with FVL plus PC, 2 with PS plus FVL, 7 with PC plus PT20210A, 6 with PS plus PT20210A, 6 with FVL plus PT20210A and 1 with AT plus PT20210A). The median age of the women who developed VTE was 28 years (range 15-49 years). Table 1 classifies the patients in regard to the type of defect and the relationship between OC intake and VTE and also includes the number of families depending on the type of thrombophilic defect. One hundred and twenty-one women had taken OC. One hundred and five women developed VTE, 60 of which were not related to OC intake. Among 220 women without VTE, 76 had taken OC. One hundred and nine women carried the PT20210A mutation, 104 in a heterozygous state and 5 in a homozygous state. Thirty-two were associated with other coagulation defects. Fifty-eight of them had taken OC. Thirty-seven carriers of this mutation had a thrombotic episode and 22 were associated with OC use. Of the 72 carriers without a history of thrombosis, 36 had taken OC. Fifty-two women were heterozygous for factor V Leiden, 15 of them in association with the PT20210A mutation and 11 with other deficits. Thirty women had VTE and 14 episodes were related to OC intake. Twenty-two women did not develop VTE and 7 of them had taken OC. Ninety-eight women had other defects, including AT, PS and PC deficiencies. Fifty-four had a thrombotic episode and 12 were associated with OC use. Forty-four of these women did not develop VTE although 7 were OC users. Information about the OC type and time of intake was obtained from only 91 women. Thirty-two of them used first-generation OC and 7 cases were associated with VTE. Twenty-nine women used second-generation OC and 6 of them had had a VTE. Third-generation OC were used by 30 women, and 15 of them had had an episode of VTE. When we analyzed the data, we observed a statistical significant difference between carriers of FVL taking first and third generation OC with non-carriers, and also between second-generation OC users of PT20210A carriers with non-carriers of this mutation.

Taking into consideration that the length of time that women are on OC treatment could affect the prevalence of VTE, we also studied the relation of duration of OC intake and the VTE episode. In all women, with or without VTE, the median duration of OC intake was 36 months (range 1-216). In VTE

Table 2. Risk of VTE in women with inherited thrombophilic factors.

	VTE (N= 105)	NO VTE (N=220)	OR*(95% CI)	OR°(95% CI)
PT20210A				
Non-carrier#	68 (64.8%)	148 (67.3%)	ref.[1]	
Carrier	37(35.2%)	72(32.7%)	1.1 (0.7-1.8)	1.9 (1.1-3.5)
Factor V Leiden				
Non-carrier#	75 (71.4%)	198 (90%)	ref.[1]	
Carrier	30 (28.6 %)	22 (10%)	3.6 (1.9-6.6)	5.7 (2.9-11.2)
Other deficiencies@				
No deficiency#	51 (48.6%)	176 (80%)	ref.[1]	
Deficiency	54 (51.4%)	44 (20%)	4.2 (2.6-7.1)	6.6 (3.7-11.8)

*VTE: venous thromboembolism. *Crude odds ratio. °OR adjusted by age, including other deficiencies, the PT20210A and the FVL mutations.@The variable "other deficiencies" includes AT, PS and PC deficiencies. #The "non-carriers" and "non-deficiency" categories include all women without the analyzed defect.*

Table 3. Risk of venous thromboembolism in patients with inherited thrombophilia using or not oral contraceptives.

	VTE with OC (N= 45)	VTE without OC (N=60)	OR* (95% CI)	OR° (95% CI)
PT20210A				
Non-carrier#	23 (51.1%)	45 (75%)	ref.[1]	
Carrier	22 (48.9%)	15 (25%)	2.9 (1.3-6.6)	3 (1.3-6.8)
Factor V Leiden				
Non-carrier#	31 (68.9%)	44 (73.3%)	ref.[1]	
Carrier	14 (31.1 %)	16 (26.7 %)	1.2 (0.5-2.9)	1.4 (0.6-3.3)
Other deficiencies@				
No deficiency#	33 (73.3%)	18 (30%)	ref.[1]	
Deficiency	12 (26.7%)	42 (70%)	0.2 (0.1-0.4)	

*VTE: venous thromboembolism. OC: oral contraceptives. *Not adjusted OR. °OR adjusted by age, including the PT20210A and the FVL mutations. The variable "other deficiencies" includes AT, PS and PC deficiencies. #The "non-carriers" and "no deficiency" categories include all women without the analyzed defect.*

associated with OC use, the median time before the VTE episode in first-generation OC users was 12 months (range 2-120), 5 months (range 2-6) in second-generation OC users and 4 months (range 2-72) in third-generation OC users.

Table 2 shows the OR obtained by conditional logistic regression for VTE for the different coagulation disorders, both as an unadjusted OR and an adjusted OR (the crude OR includes all variables and the other adjusted OR excludes the variable *other deficiencies* as a modifying factor). All subjects with inherited disorders showed a higher risk of VTE compared with non-carriers of any cause of inherited thrombophilia. Subjects with *other deficiencies* showed an adjusted OR of 6.6 (95% CI 3.7-11.8). For the statistical analysis, we included homozygous and heterozygous carriers of PT2010A and FVL mutations in the same groups, because of the small number of women homozygous for these mutations. The adjusted OR for the PT20210A and the FVL mutations were 1.9 (95% CI 1.1-3.5) and 5.7 (95%CI 2.9-11.2), respectively. When we studied women with double defects we did not find statistical differences in the risk of VTE (data not shown).

The crude OR for VTE in OC users depending on the type of inherited disorder is shown in Table 3. For carriers of the PT20210A mutation, the risk was 2.9-fold higher (95% CI 1.3-6.6) than that of non-carriers. Carriers of the FVL mutation showed an OR of 1.2 (95% CI 0.5-2.9), and in the group of women with *other deficiencies* compared with those without *other deficiencies*, the OR was 0.2 (95% CI 0.1-0.4). When we performed conditional logistic regression analysis including all of the variables, OC users with any inherited disorders except *other deficiencies*, had an increased risk of VTE. Taking into consideration that *other deficiencies* (PS, PC and AT deficiencies) are independent high risk factors in younger women, whether or not they are taking OC,[16] we decided to consider them as a modifying factor in the conditional logistic regression analysis. The variable *other deficiencies* was considered as a modifying factor because it is associated with a high risk of VTE in the presence or not of environmental factors, including OC intake.[12] VTE in patients with PC, PS or AT deficiencies are spontaneous in 50% of the cases. This indicates that changes in the environment may be less determinant than the genetic background in the risk of VTE. Also, different studies in families with these deficiencies have not shown an increased risk of VTE in OC users.[12,16] It is also important to note that these patients developed VTE at younger age,[11] and this fact may reflect a bias of *confounding by indication*, due to the advertisement to the patients and family members to discontinue OC intake. In this study, probands were included to avoid underestimating the OR and to increase the statistical power of our analysis. Age-adjusted OR did not show any difference. When we analyzed our data excluding PC, PS and AT defects (*other deficiencies* considered as a modifying factor), the risk of VTE in OC users was 1.4 (95%CI 0.6-3.3) for the FVL carriers and 3.0 (95% CI 1.3-6.8) for the PT20210A mutation carriers. We did not find a

statistically increased risk in women with double defects compared with in women with a single defect (data not shown).

Lifetime risk of thrombosis was studied with survival methods. The probability of developing VTE was significantly higher in women with inherited disorders than in women without these disorders (data not shown). When we performed the analysis depending on the inherited thrombophilic factors, only the PT20210A mutation carriers showed a statistically significant increase of VTE in OC users compared with in non-carriers. We found a three-fold increased risk of VTE in OC users who were PT20210A carriers. The lifetime risk of VTE of these patients was 2.9 (95% CI 1.2-5.3) and the median time in years to develop the episode of VTE was 35 years (95% CI 29-41).

Discussion

A number of case-control studies have been reported to establish the risk of VTE associated with OC intake.[3,5-8] Most of them have shown an increased risk of VTE in women using OC whether or not they exhibited inherited thrombophilia, although other authors had not found this increased risk. In 1994, Pabinger *et al.*[16] reported a retrospective controlled-cohort study that evaluated the thrombotic risk of women with inherited thrombophilia (AT, PC and PS deficiencies) taking OC. They found that only women with AT deficiency had a higher risk of VTE when taking OC. In 1995, Bloemenkamp *et al.*[13] found, in a case-control study, that women with the FVL mutation had a higher risk of VTE if they were using OC (in particular OC containing a third-generation progestagen). Recently, Zotz RB *et al.*[8] reported a higher risk of VTE in women taking OC with thrombophilia, including PT20210A and methylenetetrahydrofolate reductase mutation, compared within women without thrombophilia.

Many authors have emphasized the need to screen families of symptomatic carriers of thrombophilic defects to prevent the occurrence of VTE in relatives, although the different results obtained in some family studies have not always supported this policy. To our knowledge, only four family cohort studies have been reported, and they compared the thrombotic risk between patients with coagulation defects and the role of environmental factors predisposing to VTE.[19-22] They studied the incidence of VTE in family members of symptomatic *propositi* with AT, PC and PS deficiencies and the FVL mutation, but none of them reported on the relative risk of VTE in carriers of the PT20210A mutation and their relatives. Results obtained in these studies regarding the role of OC intake and VTE are controversial. Martinelli *et al.*[19] reported a higher risk of VTE in carriers of AT, PS and PC deficiencies and the FVL mutation. When environmental factors, such as OC, were considered, no differences in the distribution in the four thrombophilic defects were observed. Simioni *et al.*[20] reported an increased risk of VTE in carriers of AT, PC, PS defects associated with OC intake compared with in their non-deficient family members. Taking into account that family members of *propositi* with these defects are encouraged to discontinue OC intake, this result may indicate a bias of *confounding by prescription*. Middeldorp *et al.*[21] observed a low annual risk of VTE in individuals carrying the FVL mutation who were relatives of heterozygous *propositi* for the FVL mutation. They concluded, in view of their results, that a general policy of screening the families of all patients with the FVL mutation did not seem to be indicated. Recently, Lensen *et al.*[22] have shown that carriers of the FVL mutation using OC had a 4.9 times higher thrombosis risk than non-carriers (RR 4.9, 95%CI 1.4-17.3). No family studies have evaluated the risk of VTE in carriers of the PT20210A mutation who use OC. We have studied the risk of thrombosis in women taking OC who belonged to families with inherited thrombophilia. In this family study we also included families that were carriers of the PT20210A mutation. Unfortunately, the number of cases was too low to evaluate the effect of different regimens of OC accurately. Nevertheless, we observed that 15 women out of 30 who used third-generation OC developed VTE. In any case, further studies will be required to acquire information on this subject.

In conclusion, our cohort family study showed an increased risk of VTE in patients with inherited thrombophilia, such as AT, PS, PC deficiencies and the FVL and the PT20210A mutations. Women with AT, PS and PC deficiencies had an increased risk of VTE, although we could not establish an increased risk associated with OC use. FVL carriers using OC showed an OR of VTE of 1.4 (95%CI 0.6-3.3); this indicates that these women have a higher tendency to develop thrombosis, in accordance with data from other authors.[19,22] The PT20210A mutation carriers using OC showed a significant OR of VTE of 3 (95% CI 1.3-6.8).

Because of the high prevalence of these two mutations in the general Spanish population, (prevalence of 2% of FVL and 6.5% of PT20210A)[2,10,25] and the increased risk of VTE asso-

ciated with OC intake, genetic screening for these mutations should be considered in potential OC users belonging to families with thrombophilia.

Contributions and Acknowledgments

AS wrote the paper and was involved in the design of the study, selection of patients, analysis and interpretation of data. JM and AO were involved in analysis and interpretation of data. BM and JCS played a part in the design of the study and selection criteria. MB, IT, JMS performed laboratory analyses. All the authors revised the manuscript and contributed to its intellectual content. JF reviewed the paper and supervised the whole study.

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Potential implications for clinical practice

Asymptomatic women with a thrombophilic defect must be informed of the risk of VTE associated with OC use.[26]

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