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ORIGINAL ARTICLE

Prothrombotic conditions, oral contraceptives, and the risk of ischemic stroke

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Summary. *Background*: The role of inherited prothrombotic conditions, including factor V Leiden (FV G1691A), prothrombin G20210A, and the methylenetetrahydrofolate reductase (MTHFR) C677T genotype, in the pathogenesis of ischemic stroke is not well established. The effects of these factors may be potentiated by the use of oral contraceptives, analogous to observations in venous thrombosis. *Methods*: Patients ($n = 193$) were women aged 20–49 years with ischemic stroke. Controls ($n = 767$) were women without arterial thrombosis stratified for age, calendar year of the index event, and residence. The relative risk of ischemic stroke was estimated with unconditional logistic regression, adjusted for stratification variables. *Findings*: Factor V Leiden and MTHFR 677TT were more common in patients than in controls [odds ratio (OR): 1.8; 95% confidence interval (CI): 0.9–3.6 respectively OR: 1.5; 95% CI: 0.9–2.6]. The frequency of prothrombin G20210A was similar in cases and controls. Carriers of FV Leiden using oral contraceptives had a 11.2-fold (95% CI: 4.3–29.0) higher risk of ischemic stroke than women without either risk factor. Women with MTHFR 677TT using oral contraceptives had a 5.4-fold (95% CI: 2.4–12.0) higher risk than women without these risk factors. *Interpretation*: These data suggest that carriers of FV Leiden or MTHFR 677TT who use oral contraceptives have an increased risk of ischemic stroke. When these findings are confirmed, a cost-effectiveness analysis should indicate whether ischemic stroke could be prevented with genetic testing before the start of oral contraceptives.

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Introduction

The factor (F)V Leiden (G1691A) and prothrombin G20210A mutations are risk factors for venous thrombosis [1,2]. The risk of venous thrombosis related with FV Leiden or prothrombin G20210A is enhanced by the use of oral contraceptives [3,4]. The association of these mutations with arterial thrombosis such as ischemic stroke has not been well established, but seems to be much weaker [5,6], possibly with the exception of female smokers [7]. Another frequent genetic factor is the methylenetetrahydrofolate reductase (MTHFR) 677T variant. The homozygous form, MTHFR 677TT was found to increase the risk of arterial thrombosis in a meta-analysis [5], but not in a recent large study [8].

The use of oral contraceptives approximately doubles the risk of ischemic stroke [9,10]. The strength of this relationship depends on smoking and hypertension [9,10], and probably other, still unknown factors. A recent, small study suggests that prothrombin G20210A increases the risk of ischemic stroke related with oral contraceptives [11].

In carriers of FV Leiden, inactivation of FVa by activated protein C (APC) is greatly impaired [1], which results in a prothrombotic state. The prothrombin G20210A mutation increases the risk of venous thrombosis via elevation of prothrombin levels [2]. MTHFR is an enzyme involved in the metabolism of homocysteine. Plasma level of homocysteine is increased in carriers of the MTHFR 677T allele [12,13]. Increasing homocysteine levels are a risk factor for stroke [14–16], possibly due to endothelial dysfunction at several levels, including depression of protein C activation [16]. Interestingly, estrogens also increase the resistance to APC, particularly oral contraceptives containing third generation progestogens (desogestrel, gestodene) [17,18].

The aim of this study was firstly to investigate whether FV Leiden, prothrombin G20210A, and MTHFR 677TT are associated with the risk of ischemic stroke, and secondly whether the association is affected by the use of oral contraceptives and smoking.

Patients and methods

Study design

The Risk of Arterial Thrombosis in relation to Oral Contraceptives (RATIO) study is a multicenter, population-based case–control study. The study consists of three substudies on stroke, myocardial infarction, and peripheral arterial disease. The first phase evaluated the risk of arterial thrombosis related to the use of oral contraceptives (1990–1995) [9,19,20]. In the second phase, blood samples were drawn or buccal swabs collected for the determination of prothrombotic conditions (1998–2002). The study was approved by the medical ethics committees of the participating hospitals. All participants gave informed consent.

Study population

Eligible cases were women aged 18–49 years who were hospitalized for a first ischemic stroke in one of nine participating Dutch hospitals between 1990 and 1995. Stroke was diagnosed in all patients based on the medical history, neurologic examination as well as computed tomography (CT)- or magnetic resonance imaging (MRI)-scan by experienced neurologists in the participating centers. Exclusion criteria were transient ischemic attack (an event lasting < 24 h), hemorrhagic stroke, cerebral venous sinus thrombosis, carotid artery dissection, history of cardiovascular or cerebrovascular diseases, terminal illness, aphasia or cognitive impairment interfering with the questionnaire or not speaking Dutch. Of the 295 eligible patients admitted during the study period, 203 (69%) patients agreed to participate in the first phase of the study [9]. Further, we approached 59 additional ischemic stroke patients who presented in the University Medical Center Utrecht between 1996 and 2001, using the same inclusion and exclusion criteria. Of 262 eligible patients in the second phase, six had died, 19 refused to participate and 44 could not be located. Therefore, 193 patients (74%) were included in the present study.

A population-based control group was identified by random digit dialing between 1990 and 1995 [9]. This group was frequency matched to the first 203 cases for age, residence, and year of the stroke [9]. Eligible were women aged 18–49 years without a history of coronary heart disease, cerebrovascular event or peripheral vascular disease. A questionnaire was sent to women who were eligible as controls. A total of 925 of 1039 eligible women (response rate 89%) were included as controls in the RATIO study for the three case groups combined. DNA was obtained from 767 women (83% of the controls of the first phase).

Data collection and definitions

Patients and controls received a standardized mail questionnaire on use of oral contraceptives, smoking status, alcohol use, weight, height, physician's diagnosis and medication use for hypertension, diabetes mellitus, and hypercholesterolemia. Color photographs of boxes of all oral contraceptives were used to help women recall the specific type of oral contraceptives they had used. All questions referred to the time period preceding the index date, i.e. the date of stroke in patients and the mid-year of the same year in controls. Current use of oral contraceptives was defined as use of a contraceptive pill within 1 month before the index date. Non-use was defined as previous use or never use. Smoking was categorized into current, former, and never smoking. Current smoking was defined as having smoked at least one cigarette a day in the year before the index date. Alcohol use before the index date was categorized into never, 1–15 drinks a week, and more than 15 drinks a week, in the year before the index date. At the time of blood collection, we were able to confirm the data on hypertension, diabetes, and hypercholesterolemia. Blood pressure was measured twice in a supine position after 5 min rest and averaged. A women was classified as hypertensive when using antihypertensive medication, having a systolic blood pressure of 160 mmHg or higher, or a diastolic blood pressure of 95 mmHg or higher at the time of venepuncture.

Isolated DNA from whole venous blood or mouth swaps was analyzed for FV Leiden, prothrombin G20210A, and the MTHFR genotype with a standard polymerase chain reaction (PCR) [7]. The technician who performed DNA analyses was blinded to whether a sample was from a patient or a control.

Statistical analysis

The relative risk of ischemic stroke associated with FV Leiden, prothrombin G20210A or MTHFR 677TT was assessed as an odds ratio (OR) with 95% confidence intervals (CI) using unconditional logistic regression. Adjustments were made for the stratification variables: age, calendar year of the index event, and residence. The additional cases were assigned the highest index year of patients from the first study phase with whom the controls were matched. The effect of a combination of risk factors, i.e. a coagulation defect and use of oral contraceptives was analyzed by computing ORs in subjects with either one or both of these risk factors, compared to those with neither risk factor, in a model adjusting for the stratification variables.

Results

Table 1 summarizes the characteristics of the 193 cases with ischemic stroke and 767 controls. The age of the patients varied between 20 and 49 years. Control women were between 18 and 53 years. The characteristics of the ischemic stroke cases were consistent with previous studies in that hypertension, diabetes

Table 1 Characteristics of the study population*

	Patients ($N = 193$)	Controls ($N = 767$)
Age†	38.6 (8.0)	39.7 (7.7)
Caucasian ethnicity	95.9 (185)	94.3 (723)
Hypertension	32.1 (62)	6.1 (47)
Diabetes mellitus	4.1 (8)	1.3 (10)
Hypercholesterolemia	7.8 (15)	2.9 (22)
Oral contraceptives use	52.3 (101)	35.7 (272)
Smoking, current	34.7 (67)	32.2 (247)
Former	44.0 (85)	34.8 (267)
Never	21.2 (41)	32.3 (248)
Alcohol use, never	46.6 (90)	29.6 (227)
0–15 drinks a week	44.6 (86)	65.2 (500)
>15 drinks a week	1.0 (2)	4.2 (32)

*Values are mean (SD) or proportions (numbers).
†Age at index date.
Data were missing on ethnicity in one patient and in four controls, on diabetes mellitus in three controls, on hypercholesterolemia in five controls, on oral contraceptive use in six controls, on smoking in five controls, and on alcohol use in 15 patient and eight controls.

mellitus, hypercholesterolemia, use of oral contraceptives, and smoking were more frequent than in the controls (Table 1). The use of oral contraceptives was associated with a 3.1-fold (95% CI: 2.0–4.6) increased risk of ischemic stroke. Cases reported less alcohol use than controls.

As shown in Table 2, FV Leiden and MTHFR 677TT were more common in patients with ischemic stroke than in controls. FV Leiden increased the risk of ischemic stroke 1.8-fold (95% CI: 0.9–3.6). The OR for ischemic stroke associated with MTHFR 677TT was 1.5 (95% CI: 0.9–2.6). Although confounding is essentially not possible for genetic risk factors, we adjusted for other risk factors for stroke, such as: hypertension, diabetes mellitus, hypercholesterolemia, smoking, use of alcohol, and oral contraceptives. This did not lead to an attenuation of the observed associations (data not shown). The frequency of prothrombin G20210A was similar in patients and controls (OR: 1.0; 95% CI: 0.3–3.0).

In women who did not use oral contraceptives, FV Leiden was not associated with an increased risk (Table 3). In the absence of FV Leiden, we found a 2.6-fold (95% CI: 1.7–4.0) increased risk of ischemic stroke for oral contraceptives users. Carriers of FV Leiden who used oral contraceptives had a 11.2-fold increased risk of ischemic stroke (95% CI: 4.3–29.0), which did not change when adjustments were made for other risk factors mentioned above. Table 3 further shows the effects of FV Leiden and smoking on the risk of ischemic stroke. The highest relative risk was found in current smokers with FV Leiden (OR: 6.3; 95% CI: 1.3–31.1) when compared with non-smoking non-carriers.

Table 2 Association of factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase (MTHFR) 677TT with ischemic stroke*

	Patients	Controls	Odds ratio
Factor V Leiden	7.8% (14/179)	5.5% (42/763)	1.8 (0.9–3.6)
Prothrombin G20210A	2.7% (5/188)	2.4% (18/763)	1.0 (0.3–3.0)
MTHFR 677TT	13.5% (26/193)	9.0% (69/764)	1.5 (0.9–2.6)

*Values are number of patients/controls and odds ratios with 95% confidence interval adjusted for age at index date, index year, and residence.
Data were missing on factor V Leiden in four controls and 14 cases, on prothrombin G20210A in four controls and five patients, and on MTHFR in three controls.

Table 3 Association of factor V Leiden (FVL) and ischemic stroke: effects of oral contraceptives and smoking*

	FVL absent	FVL present
No oral contraceptives	83/463	2/24
	1 (reference)	0.4 (0.1–1.9)
Oral contraceptives	82/254	12/16
	2.6 (1.7–4.0)	11.2 (4.3–29.0)
Never or former smokers	109/477	10/35
	1 (reference)	1.6 (0.7–3.6)
Current smokers	56/242	4/5
	1.0 (0.7–1.5)	6.3 (1.3–31.1)

*Values are number of patients/controls and odds ratios with 95% confidence interval adjusted for age at index date, index year, and residence.
Data were missing on FVL in four controls and 14 cases, on oral contraceptive use in six controls and on smoking in five controls.

The combined effects of MTHFR 677TT and the use of oral contraceptives are shown in Table 4. There was no increased risk of ischemic stroke associated with MTHFR 677TT in women who did not use oral contraceptives. Oral contraceptives increased the risk of ischemic stroke in the absence of MTHFR 677TT (OR: 2.8; 95% CI: 1.8–4.3), and apparently more so in the presence of MTHFR 677TT (OR: 5.4; 95% CI: 2.4–12.0). These observations were not affected by additional adjustments for the above-mentioned risk factors (data not shown). The combined effects of smoking and MTHFR 677TT did not exceed the separate effects (Table 4). As there were only five patients who carried the prothrombin G20210A mutation, we could not study the combined effects with oral contraceptives.

Table 4 Association of methylenetetrahydrofolate reductase (MTHFR) C677T and ischemic stroke: effects of oral contraceptives and smoking*

	CC or CT	TT
No oral contraceptives	82/441	10/46
	1 (reference)	1.1 (0.5–2.4)
Oral contraceptives	85/248	16/23
	2.8 (1.8–4.3)	5.4 (2.4–12.0)
Never or former smokers	110/472	16/41
	1 (reference)	1.4 (0.7–2.8)
Current smokers	57/219	10/28
	1.0 (0.7–1.5)	1.7 (0.7–4.1)

*Values are number of patients/controls and odds ratios with 95% confidence interval adjusted for age at index date, index year, and residence.
Data were missing on MTHFR in three controls, on oral contraceptive use in six controls and on smoking in five controls.
CC, CT and TT denote the MTHFR C677T genotypes.

Discussion

In this population-based, case–control study of women of reproductive age, we found that FV Leiden and MTHFR 677TT increased the risk of ischemic stroke. Furthermore, we found a high relative risk of ischemic stroke for both FV Leiden and MTHFR 677TT in combination with the use of oral contraceptives. In addition, smoking carriers of FV Leiden had an increased risk of ischemic stroke relative to women without either risk factor.

In the interpretation of these findings, a possible limitation is that we only studied women who survived an ischemic stroke. However, mortality after stroke in young women is low [21]. Indeed, of the eligible patients in this study, mortality was 6% ($n = 18$) before inclusion in the first phase [9], and 2% ($n = 6$) before the second wave. If the genetic factors of interest would have been associated with case-fatality, selection of surviving ischemic stroke patients would have led to an underestimation of the true effect. Secondly, we did not collect data on the subtype of ischemic stroke. The proportion of patients with a cardioembolic stroke is likely to be low, because history of cardiovascular disease was used as an exclusion criterion. Thirdly, we included hospitalized patients only. However, as a stroke is uncommon before the age of 50 years [22], it is unlikely that patients of this age will not be referred to the hospital and thus not included in this study. The control group was population-based with a high response rate, and these women were not informed about the determinants of this study. It is therefore not likely that the willingness to participate was related to determinants under study, such as the use of oral contraceptives. All laboratory analyses were performed without knowledge of case–control status. Inaccurate information on oral contraceptive use is unlikely as we used color photographs of pillboxes to determine exposure to oral contraceptives. Based on these considerations, we think it is unlikely that our findings are biased.

An advantage of this study is its large size, for a disease that is infrequent in young women. As the use of oral contraceptives in the Netherlands is high (approximately 35%), we were able to study joint effects with FV Leiden and MTHFR 677TT. Further, we included hospitalized patients only and excluded transient ischemic attacks. As diagnostic procedures in these young women were extensive, it is unlikely that incorrect diagnosis of stroke has played an important role. We did not explore subtypes of ischemic stroke, as this study aimed to investigate the implications for public health of oral contraceptives and prothrombotic mutations.

The risk of arterial thrombosis associated with FV Leiden, prothrombin G20210A or MTHFR 677TT may dilute with aging, and seems to be higher in women than in men [5,6,23–25]. Compared with previous investigations, our study population was relatively young and consisted entirely of women. This may explain that we detected higher ORs for ischemic stroke than most other studies [5,6,8,23]. The relatively high proportion of oral contraceptives users could also explain differences across studies.

Our study suggests a high relative risk for combinations of FV Leiden as well as MTHFR 677TT with oral contraceptives in the risk of ischemic stroke. Our findings should be replicated in other studies and followed by a cost-effectiveness analysis before large-scale genetic screening is considered.

Contributors

A Algra, FR Rosendaal, and Y van der Graaf designed the study. AJC Slooter, A Algra, FR Rosendaal obtained funding. BC Tanis, JM Kemmeren, and AJC Slooter were responsible for acquisition of data, supervised by A Algra, FR Rosendaal, and Y van der Graaf. AJC Slooter analyzed and interpreted the data, under supervision of A Algra and FR Rosendaal. AJC Slooter wrote the first draft of the manuscript, to which the other investigators added their comments. All authors approved the final version of the manuscript.

Conflict of interests

None declared.

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