

Coagulation factor XIII gene variation, oral contraceptives, and risk of ischemic stroke

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Prothrombotic conditions are associated with ischemic stroke in young women. In particular, the combination of oral contraceptive use and prothrombotic genetic variants appears to increase the risk of ischemic stroke. We performed a population-based case-control study in 190 women aged 20 to 49 years with ischemic stroke and 767 women without cardiovascular disease stratified for age, calendar year of the index event, and residence. A total of 4 variants of coagulation factor XIII subunit A and B genes

(*F13A1* and *F13B*) were investigated. The Phe allele of the *F13A1* Tyr204Phe variant was present in 59 (31%) patients and 43 (6%) controls; the odds ratio for ischemic stroke was 9.1 for Phe/Phe and Phe/Tyr versus Tyr/Tyr genotype; the 95% confidence interval was 5.5 to 15. Homozygous genotypes (Phe/Phe) conferred a higher risk (odds ratio, 77; 95% confidence interval, 7.0-848) than heterozygous (Tyr/Phe) genotypes (odds ratio, 8.2; 95% confidence interval, 4.9-14). The risk of ischemic stroke was further increased

in carriers of the 204Phe allele using oral contraceptives (odds ratio, 20; 95% confidence interval, 9-46) compared with non-users with Tyr/Tyr genotype. In conclusion, the *F13A1* 204Phe allele was strongly associated with ischemic stroke in young women. Oral contraceptive use further increased the risk of ischemic stroke. (Blood. 2008;111:1282-1286)

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Introduction

Prothrombotic conditions are associated with ischemic stroke in young women. For example, oral contraceptive use approximately doubles the risk of ischemic stroke.¹ Genetic factors also appear to play a role as is indicated by familial aggregation of ischemic stroke in young women.² Two prothrombotic genetic variants, the prothrombin G20210A variant and the methylenetetrahydrofolate (MTHFR) C677T variant, have been associated with ischemic stroke in young women.³ Previous studies also found evidence of a combined effect of oral contraceptive use and prothrombotic gene variation on ischemic stroke risk.³⁻⁶

Coagulation factor XIII (FXIII) is a protransglutaminase that circulates as a heterotetramer composed of 2 A and 2 B subunits. Activated FXIII is involved in cross-linking of the fibrin clot by transglutaminase reactions between glutamine and lysine residues on fibrin. The effect of a specific change in FXIII plasma level or activity on clot structure and thrombotic risk appears to depend upon several other factors, including the concentrations of thrombin, prothrombin, fibrinogen, and fibronectin.⁷⁻⁹ A twin study showed a clear genetic influence on fibrin clot structure.¹⁰ Several variants in the FXIII subunit A and B genes (*F13A1* and *F13B*) change the FXIII level or activity and may therefore affect clot structure and the risk of thrombotic disease. Some of these variants have also been previously associated with myocardial infarction or ischemic stroke. The Leu allele of the *F13A1* Val34Leu variant (Ref SNP ID no.: rs5985) increases the rate of FXIII activation and alters fibrin clot structure,¹¹ and has been associated with a

decreased risk of myocardial infarction¹² and ischemic stroke.¹³ The Phe allele of the *F13A1* Tyr204Phe variant (rs3024477) lowers both FXIII plasma level¹⁴ and FXIII activity,¹⁵ and slightly increased the risk of ischemic stroke in young women in 1 study.¹⁶ The Leu allele of the *F13A1* Pro564Leu variant (rs5982) also lowers FXIII plasma levels¹⁴ but increases FXIII activity.¹⁵ The Pro564Leu variant was not associated with ischemic stroke or myocardial infarction in young women in a previous study.¹⁶ The Arg allele of the *F13B* His95Arg variant (rs6003) increases FXIII subunit dissociation¹⁷ and may reduce the risk of myocardial infarction in postmenopausal women.¹⁸ The relationship between the His95Arg variant and ischemic stroke has not yet been studied.

We hypothesized that 4 specific functional variants in *F13A1* and *F13B* may be associated with ischemic stroke in young women. Because oral contraceptive use and smoking also affect hemostasis,¹⁹ we also studied their effects on the association of genetic variation in FXIII and ischemic stroke.

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 3 substudies on ischemic stroke, myocardial infarction, and peripheral arterial disease. The first phase evaluated the risk of arterial

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thrombosis related to the use of oral contraceptives (1990-1995).²⁰ In the second phase, blood samples were drawn or buccal swabs collected to study prothrombotic conditions (1998-2002). The study was approved by the medical ethics committees of the participating hospitals.

Patients

Eligible patients were women aged 18 to 50 years who were hospitalized for a first ischemic stroke in 1 of 9 participating Dutch hospitals between 1990 and 1995. Stroke was diagnosed in all patients based on the medical history and 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 less than 24 hours), hemorrhagic stroke, cerebral venous sinus thrombosis, carotid artery dissection, history of cardiovascular or cerebrovascular disease, 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 patients agreed to participate in the first phase of the study.²⁰ A population-based control group was identified by random-digit dialing between 1990 and 1995.²⁰ This group was frequency-matched to the patients for age, residence, and year of the stroke. Eligible controls were women aged 18 to 50 years without a history of coronary heart disease, cerebrovascular event, or peripheral vascular disease. A total of 925 of 1039 eligible women were included as controls in the RATIO study for the 3 patient groups combined. DNA was obtained from 767 women. Of the 203 patients from the first phase, 140 patients also participated in the second phase: 6 had died, 10 refused to participate, 44 did not respond, and 3 had insufficient availability of DNA. To compensate for the loss of patients, we approached 59 additional patients with ischemic stroke who presented in the University Medical Center Utrecht between 1996 and 2001 using the same inclusion and exclusion criteria. Of these 59 patients, 50 were included (9 refused to participate). Therefore, 190 patients participated in the present study. Informed consent was obtained in accordance with the Declaration of Helsinki.

Data collection and definitions

Patients and controls received a standardized mail questionnaire between 1997 and 2001 on use of oral contraceptives, smoking status, alcohol use, weight, height, physician's diagnosis, and medication use for hypertension, diabetes mellitus, and hyperlipidemia. Color photographs of boxes of all oral contraceptives marketed in the Netherlands 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 (ie, 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. Nonuse was defined as previous use or never having used. Smoking was categorized into current, former, and never having smoked. Current smoking was defined as having smoked at least 1 cigarette a day in the year before the index date. Alcohol use before the index date was categorized into never, 1 to 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 hyperlipidemia. Blood pressure was measured twice in a supine position after 5 minutes of rest and averaged. A woman was classified as hypertensive when using antihypertensive medication, having a systolic blood pressure of 160 mmHg or higher, or having a diastolic blood pressure of 95 mmHg or higher at the time of blood collection.

DNA was isolated from 10-mL EDTA-augmented blood or buccal swabs and amplified by polymerase chain reaction technique. Blood samples were available for 175 patients and 638 controls. Buccal swabs were collected in 15 patients and 129 controls. A total of 4 genetic variants were genotyped with the 5' nuclease/TaqMan assay:²¹ Val34Leu (rs5985), Tyr204Phe (rs3024477), and Pro564Leu (rs5982) variants in the FXIII subunit A gene and the His95Arg variant (rs6003) in the FXIII subunit B gene. No other variants were genotyped. Polymerase chain reactions with

Table 1. Baseline characteristics of young women with ischemic stroke and control women

	Patients	Controls
No.	190	757
Age, y*	39.8	38.6
European ethnicity, no. (%)	182 (96)	723 (94)
Hypertension, no. (%)	62 (33)	47 (6)
Diabetes mellitus, no. (%)	8 (4)	10 (1)
Hyperlipidemia, no. (%)	15 (8)	22 (3)
Oral contraceptive use, no. (%)	98 (52)	272 (36)
Smoking, no. (%)		
Current	66 (35)	247 (32)
Former	85 (45)	272 (36)
Never	39 (21)	248 (32)
Alcohol use, no. (%)		
Never	89 (47)	227 (30)
0-15 drinks a week	84 (44)	500 (65)
> 15 drinks a week	2 (1)	32 (4)

Data were missing on ethnicity in 1 patient and in 4 controls, on diabetes mellitus in 3 controls, on hyperlipid in 5 controls, on oral contraceptive use in 6 controls, and on alcohol use in 15 patients and 8 controls.

*Mean age at index date.

fluorescent allele-specific oligonucleotide probes (Assay-by-Design/Assay-on-Demand; Applied Biosystems, Foster City, CA) were performed on a PTC-225 thermal cycler (Biozym, Hessisch Oldendorf, Germany), and fluorescence end-point reading for allelic discrimination was performed on an ABI 7900 HT (Applied Biosystems). Primer sequences, probe sequences, and restriction enzymes used are available on request. The reference cluster ID (rs) numbers for genetic variants (also included in Table 2) enable retrieval through the dbSNP website.²² The laboratory technicians were unaware of case-control status and other patient characteristics.

Statistical analysis

The relative risk of ischemic stroke associated with genetic variation in FXIII was assessed as an odds ratio with a 95% confidence interval using unconditional logistic regression. A dominant model of inheritance was used: variant allele heterozygotes and homozygotes were compared with wild-type homozygotes. Adjustments were made for the stratification variables: age, calendar year of the index event, and residence. The additional patients were assigned the highest index year of patients from the first study phase with whom the controls were matched. Odds ratios adjusted for cardiovascular risk factors were not computed because environmental factors are unlikely to affect genotype frequencies and to confound genetic associations.²³ The combined effect of oral contraceptive use and genetic variation in FXIII on ischemic stroke was analyzed by computing odds ratios in patients with either one or both of these risk factors, as compared with those with neither risk factor, in a model adjusting for the stratification variables. The combined effect of smoking and genetic variation in FXIII on ischemic stroke was analyzed similarly. Odds ratios involving oral contraceptive use were adjusted for stratification variables and vascular risk factors. The genotype distribution in the control group was in Hardy-Weinberg equilibrium and similar to the genotype distribution for Europeans in the HapMap project²² for all tested genetic variants.

Results

Table 1 provides baseline characteristics of 190 women with ischemic stroke and 767 control women. The age of the patients ranged from 20 to 50 years, and the age of the control women varied from 18 to 53 years. Risk factors for ischemic stroke

Table 2. Genotype distribution in patients and controls and odds ratios for ischemic stroke under a dominant genetic model

Gene name (HUGO abbreviation)	Variant	RefSNP ID no.	Distribution in patients (%) (n = 190)			Distribution in controls (%) (n = 767)			Odds ratio (95% CI)*
			AA	AB	BB	AA	AB	BB	
Factor XIII subunit A (<i>F13A1</i>)	Val34Leu	rs5985	121 (64)	60 (32)	8 (4)	419 (56)	283 (38)	45 (6)	0.77 (0.53-1.11)
Factor XIII subunit A (<i>F13A1</i>)	Tyr204Phe	rs3024477	116 (66)	54 (31)	5 (3)	711 (94)	42 (6)	1 (0)	9.1 (5.5-15)
Factor XIII subunit A (<i>F13A1</i>)	Pro564Leu	rs5982	107 (62)	57 (33)	8 (5)	466 (62)	251 (33)	34 (5)	0.89 (0.61-1.29)
Factor XIII subunit B (<i>F13B</i>)	His95Arg	rs6003	134 (76)	33 (19)	10 (6)	609 (83)	112 (15)	9 (1)	1.7 (1.1-2.7)

HUGO indicates Human Genome Organization; RefSNP ID no., reference single nucleotide polymorphism identification number; 95% CI, 95% confidence interval; AA, wild-type homozygote; AB, variant heterozygote; and BB, variant homozygote. Odds ratios are adjusted for age at index date, index year and residence. The total number of genotypes for each genetic variant does not add up to the total number of subjects because genotype was undetermined in some subjects. Percentages do not always add up to 100% due to rounding.

* Odds ratio with 95% confidence interval assuming dominant inheritance: heterozygous (AB) and homozygous (BB) variant genotypes versus wild-type (AA).

(hypertension, diabetes mellitus, hyperlipidemia, oral contraceptive use, and smoking) were more common in patients than in controls. Patients reported less alcohol use than controls. After adjustment for stratification variables and vascular risk factors, oral contraceptive use increased the risk of ischemic stroke (odds ratio, 2.7; 95% confidence interval, 1.7-4.1). All oral contraceptives were combinations of an estrogen (ethinyl estradiol) and a progestin. The estrogen dose ranged between 20 and 50 μ g. Most oral contraceptives contained 30 μ g estrogen (n = 192; 52%). The most common types of progestin used were levonorgestrel (n = 177; 48%), desogestrel (n = 83; 22%), lynestrenol (n = 22; 6%), and cyproterone acetate (n = 20; 5%). The estrogen dose and the type of progestin were evenly distributed in patients and controls.

The overall call rate for all 4 genetic variants was 96.5% (range, 94.8%-97.8%). Genotype distribution in patients and controls and odds ratios for ischemic stroke are shown in Table 2. Carriers of at least 1 Phe allele of the *F13A1* Tyr204Phe variant had a 9-fold increased risk of ischemic stroke. Subjects with a homozygous genotype (Phe/Phe) had a much higher risk of ischemic stroke (odds ratio, 77; 95% confidence interval, 7.0-848) than subjects with wild-type (Tyr/Tyr). Subjects with a heterozygous genotype (Tyr/Phe) also had a higher risk than subjects with wild-type (odds ratio, 8.2; 95% confidence interval, 4.9-14). The *F13B* His95Arg variant conferred a 1.7-fold increased risk of ischemic stroke. The risk was higher for homozygote (Arg/Arg) carriers (odds ratio, 6.2; 95% confidence interval, 2.1-18) than for heterozygote (His/Arg) carriers (odds ratio, 1.4; 95% confidence interval, 0.87-2.3). The Leu allele of the *F13A1* Val34Leu variant slightly reduced (23%) the risk of ischemic stroke.

In oral contraceptive users who carried the 204Phe allele, the risk of ischemic stroke was increased 20-fold compared with that of nonusers with the Tyr/Tyr genotype (Table 3). No such combined effect was found between oral contraceptive use and the other 3 genetic variants. We also found no combined effect between smoking status and genetic variation in FXIII on the risk of

ischemic stroke. A combined effect of the 95Arg allele and the 34Leu allele was also absent.

Discussion

The Phe allele of the Tyr204Phe variant of the coagulation FXIII subunit A gene was associated with a 9-fold increased risk of ischemic stroke in this population-based case-control study among young women. The heterozygous and homozygous genotype increasingly affected stroke risk (8-fold and 77-fold, respectively), indicating a gene-dose effect compatible with an additive mode of inheritance. Oral contraceptive use combined with carriage of the 204Phe allele led to a 20-fold increased risk of ischemic stroke. The Arg allele of the *F13B* His95Arg variant was slightly associated with ischemic stroke but showed no combined effect with oral contraceptive use. A gene-dose effect was also observed for the 95Arg allele. The *F13A1* Val34Leu and Pro564Leu variants did not show an association with ischemic stroke or combined effect with oral contraceptive use or smoking. We could not replicate (1) a previously reported increased risk of ischemic stroke by the Leu allele of the Val34Leu variant in young women¹⁶ and (2) a combined effect of the 95Arg allele and the Leu allele of the Val34Leu variant.¹⁸

The strong association we found between the 204Phe allele and ischemic stroke is biologically plausible because this allele has been shown to lower both the FXIII plasma level¹⁴ and FXIII activity.¹⁵ A lower FXIII plasma level and activity can theoretically lead to both an increased or decreased thrombotic risk depending on several other factors, including the concentrations of thrombin, prothrombin, fibrinogen, and fibronectin.⁷⁻⁹ Since familial aggregation of ischemic stroke is higher in young women,² stronger genetic effects on stroke can be expected than in an elderly population. The 204Phe allele was associated with a slightly increased risk of ischemic stroke in young women

Table 3. Effect of oral contraceptive use on the association of the Tyr204Phe variant of the coagulation FXIII subunit A gene and ischemic stroke

Oral contraceptive use	204Pheallele	Distribution in patients, %			Distribution in controls, %		Odds ratio (95% CI)*
		Tyr/Tyr	Tyr/Phe	Phe/Phe	Tyr/Tyr†	Tyr/Phe	
Absent	Absent	54	—	—	455	—	1 (reference)
Present	Absent	62	—	—	250	—	2.6 (1.6-4.3)
Absent	Present	—	29	2	—	26	8.8 (4.3-18)
Present	Present	—	25	3	—	16	20 (9-46)

— indicates not applicable.

*Odds ratios with 95% confidence interval assuming dominant inheritance (Tyr/Phe and Phe/Phe variant genotypes versus Tyr/Tyr) adjusted for age at index date, index year, residence, hypertension, diabetes, hyperlipidemia, and smoking.

†Data on oral contraceptive use was missing in 6 controls.

(odds ratio, 1.95; 95% confidence interval, 0.63-6.05) in 1 smaller previous study.¹⁶ The 204Phe allele was not associated with myocardial infarction in this previous study (odds ratio, 1.02; 95% confidence interval, 0.33-3.14).¹⁶ The prevalence of the 204Phe allele in control women in our study (6%) is similar to the prevalence reported in this previous study (6.1%).¹⁶

Our study design has strengths and limitations. Women with a fatal ischemic stroke were not included. However, fatal ischemic stroke is rare in young women,²⁴ and not including fatal ischemic strokes is unlikely to have led to major bias.

Second, we included hospitalized patients only. Since ischemic stroke in young women is rare and requires an extensive diagnostic workup, it is unlikely that they would not be referred to a hospital and thus would not be included in this study. The chance of an incorrect diagnosis of ischemic stroke is also minimal due to the extensive diagnostic workup and the exclusion of transient ischemic attacks. Moreover, all medical records were reviewed for the purpose of this study by a neurologist.

We cannot comment on the association of FXIII plasma levels or other relevant markers of functionality (such as fibrin clot structure) with the 204Phe allele in our population that would provide evidence for causality of the association of the 204Phe allele with ischemic stroke. The RATIO study was not originally designed to study such associations. Additional functionality studies are needed to confirm the relation between the Tyr204Phe variant, hemostatic markers, and ischemic stroke in young adults. The effects of oral contraceptive use (in the presence and absence of the 204Phe allele) on FXIII plasma levels and other relevant hemostatic markers also merit further study. If the association with the 204Phe allele is replicated, the possibility that the 204Phe allele is not the causal variant needs to be considered. The 204Phe allele may be in high linkage disequilibrium with the true causal variant: coinheritance of the 204Phe allele with a truly causal variant (located in close proximity on the gene) would then lead to the association of the 204Phe allele with ischemic stroke.

Oral contraceptive use in both patients and controls was more common in our study than in 3 other genetic studies (1 Spanish and 2 Italian),^{3,4,6} which is in line with the geographic variation in prevalence of oral contraceptive use.²⁵ The relation between ischemic stroke, oral contraceptives, and genetic variation in FXIII has not been studied previously, and replication in another population of young women is needed to confirm the association we found.

A strength is the recruitment of a population-based control group by random-digit dialing with a high response rate. Control women were not informed about the aim of the study, which makes a relation between willingness to participate and determinants under study unlikely. The participants' recall was optimized by the inclusion in the questionnaire of color photographs of all available oral contraceptives.²⁶ However, the possibility of recall bias cannot be excluded.

Another strength is the large study size for a disease that is rare in young women. We were able to study a well-defined heritable phenotype in an ethnically homogeneous nationwide Dutch population. Generalizability of our findings to other ethnicities is uncertain. However, a recent meta-analysis showed genetic associations with ischemic stroke to be similar among study populations of Asian and European descent, suggesting differential genetic effects by ethnicity may be limited.²⁷

We found a 20-fold increased risk of ischemic stroke for carriership of the 204Phe allele combined with oral contraceptive use. Previous studies similarly found an increased risk of ischemic stroke for other prothrombotic gene variants combined with contraceptive use: 11-fold for factor V Leiden,⁵ and 5- to 12-fold for the MTHFR 677TT genotype.^{3,5} However, there is no indication for screening for prothrombotic genetic variants in current clinical practice.²⁸ If these genetic associations are replicated, a cost-effectiveness analysis should be performed that takes into account the low incidence of ischemic stroke among young women. The incidence of ischemic stroke among young women is reported to be 8.5 per 100 000 per year in the Netherlands.²⁹ In our study, carriers of the 204Phe allele had a 8.8-fold increased risk of ischemic stroke and a 20-fold increased risk in combination with oral contraceptive use (Table 3). Based on these data, the estimated "number needed to harm" would be 2324, meaning 1 ischemic stroke would be prevented if 2324 women carrying the 204Phe allele would refrain from using oral contraceptives for 1 year. Assuming a 6% prevalence of the 204Phe allele, more than 40 000 young women would need to be screened to prevent 1 ischemic stroke each year. Moreover, the benefits of oral contraceptive use, such as a reduction in ovarian cancer risk,³⁰ endometrial cancer risk,³¹ and pregnancy-associated morbidity and mortality,³² must also be considered. Type of progestin and estrogen dose may also affect the risk of ischemic stroke oral contraceptive users who carry the 204Phe allele. We refrained from subgroup analyses taking these factors into account. Such subgroups would be too small to detect an association with sufficient precision. The risks of other thrombotic diseases, like deep venous thrombosis and myocardial infarction, in oral contraceptive users who carry the 204Phe allele have not yet been studied.

In conclusion, we found a strong association between the 204Phe allele of the coagulation FXIII subunit A gene and the risk of ischemic stroke in young women. Oral contraceptive use further increased the risk of ischemic stroke.

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Authorship

Contribution: A.J.C.S., F.R.R., Y.v.d.G., and A.A. designed the research; D.M.O.P. and A.A. analyzed the data and drafted the manuscript. All authors contributed to interpretation of the data and critical revision of the manuscript.

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