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IN FOCUS

Factor V Leiden and prothrombin G20210A polymorphisms as risk factors for miscarriage during a first intended pregnancy: the matched case–control 'NOHA first' study

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Summary. Factor V Leiden (FVL) and prothrombin G20210A (FIIG20210A) mutations are associated with a higher risk of miscarriage: we sought to understand whether this association differs by clinical time of unexplained miscarriage, and by ethnic origin, among women with no previous thrombotic episode, during the first intended pregnancy. We performed a case–control study nested in a cohort of 32 683 women. We analyzed 3496 pairs of women matched for classical confounding factors. The FVL and FIIG20210A mutations were associated with an increased risk of miscarriage in Caucasian women [odds ratio (OR) 3.19, 95% confidence interval (CI) 2.37–4.30, $P < 0.001$ and OR 2.36, 95% CI, 1.72–3.24, $P < 0.001$, respectively]. Among non-Caucasian women, the mutations were rare and the associations with risk of miscarriage less clear. FVL and FIIG20210A mutations were independent risk factors for miscarriages only for women with related clinical signs occurring from the 10th week of gestation on (OR 3.46, 95% CI 2.53–4.72, $P < 0.001$ and OR 2.60, 95% CI 1.86–3.64, $P < 0.001$, respectively). These results indicate that FVL and FIIG20210A mutations are associated with a significant risk of spontaneous abortion which clinical signs occur from the 10th week on of the first intended pregnancy.

Keywords: factor V Leiden polymorphism, miscarriage, pregnancy loss, prothrombin G20210A polymorphism.

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Introduction

Unexplained pregnancy loss is a common health problem. A number of cohort and case–control studies performed during the 1990s examined the relationship between miscarriage and inherited hypercoagulable disorders that promote thrombosis, collectively termed 'inherited thrombophilia'; the two most common being the factor V Leiden (FVL) and prothrombin gene G20210A (FIIG20210A) mutations.

The results of three published meta-analyses [1–3] globally support an epidemiological link between maternal FVL or FIIG20210A genotypes and pregnancy loss, with variations in the magnitude of the association according to type of pregnancy loss (primary vs. secondary, isolated vs. recurrent, or first trimester vs. second and/or third trimester). However, a recent cohort study indicates that maternal thrombophilias may not be associated with pregnancy wastage prior to the 10th week of gestation, when the uteroplacental circulation is not yet functional [4].

No particular analysis has been done during a first intended pregnancy concerning the risk of unexplained pregnancy loss that may be associated with FVL and FIIG20210A maternal mutations. One of the available meta-analyses found that race had a significant influence on the association between FVL and recurrent pregnancy loss [2]. Therefore, we performed a specific large-matched case–control study, giving special attention to the ethnic origin of the women and to the timing of pregnancy loss.

Methods

Cases and controls

The Nimes Obstetricians and Haematologist study on first pregnancy, named the 'NOHA First' study, was designed

in order to perform a matched case–control analysis on 3500 patients with an unexplained pregnancy loss during their first intended pregnancy. The study began on January 1, 1999 and took place in the geographic region of southern France whose inhabitants depend on the University Hospital of Nimes (Nimes basin). This included roughly 1 million people principally living in the administrative subdivision of the Gard (625 000 inhabitants) or living close to the boundaries of this subdivision in the bordering towns of Hérault, Aveyron, Lozère, Ardèche, Drôme, Vaucluse and Bouches-du-Rhône. The annual number of births for this population is approximately 11 000.

For cases and controls, thrombophilic conditions, like for instance clotting inhibitor deficiencies or antiphospholipid/cofactor antibodies, were not assessed prior to selection and were not used as inclusion or exclusion criteria.

Selection of case subjects

A total of 32 683 women experiencing their intended first pregnancy were followed-up by a network of 37 gynecologists and obstetricians distributed throughout the whole region. This network included representatives of gynecologists and obstetricians working in general hospitals and clinics of the Nimes basin, and at the University Hospital of Nimes. Each member of this network worked with their usual general practitioner to keep informed of all women experiencing a first pregnancy for the ongoing study.

These women were advised to have a blood pregnancy test 1 week after a missed menstrual period or as soon as they believed that they were pregnant.

We recorded all the cases of pregnancy loss that occurred in this cohort (Fig. 1). The gestational age at the time of spontaneous abortion was calculated on a pure clinical basis, as the time between the date of the first day of the last normal menstrual period, and between the date of the first clinical symptom related to pregnancy loss.

The final cases were women who satisfied the inclusion and exclusion criteria, who signed the written informed consent to participate in the study, for the collection of blood and subsequent DNA analysis, and whose male partners also gave their written informed consent in order to create a specific plasma and DNA bank for further studies.

The inclusion criteria were an unexplained pregnancy loss during the first intended pregnancy. Each of the putative cases was examined by the members of the network for any known underlying risk factors according to an invariable protocol that had been previously proposed by the medical staff of the Department of Gynaecology and Obstetrics, University Hospital, Nimes. This included screens for: infectious diseases during pregnancy (systematic HIV, HCV, HBV and *Chlamydia trachomatis* serologies); chromosomal abnormalities (karyotype of both parents and of the abortus); uterine anatomical abnormalities (hysterosalpingoscopy); toxoplasmosis serology; diabetes mellitus; thyroid function and thyroid antibodies even in the absence of abnormal thyroid function (antithyroglobulin and antithyroid peroxydase antibodies); hyperprolactinaemia prior to luteal phase defects (a normal luteal phase of at least 12 days and plasma progesterone above 25 ng mL^{-1}); antinuclear (indirect immunofluorescence of Hep-2 cells), anti-dsDNA (Farr assay), and antiextractable nuclear antigens antibodies; classical antiphospholipid antibodies (plasma lupus anticoagulant activity, anticardiolipin, and anti-β2-glycoprotein I IgG and IgM antibodies according to previously described methods [5]); erythroblastosis fetalis -Rh disease-; immune thrombocytopenic purpura; and fetomaternal alloimmune thrombocytopenia. Any data missing from this protocol led to the patient being excluded from the study. Any abnormality identified led to the pregnancy loss being defined as explained.

Firstly, the exclusion criteria were explained pregnancy loss; secondly, any previous personal occurrence of superficial or deep vein thrombosis, or in any first degree relative, because a consistent number of these women had already been investigated for thrombophilia prior to this study; thirdly, any treatment during pregnancy interfering with the hemostatic system, including low- or high-dose aspirin; and pre-eclampsia, defined as gravidic hypertension [systolic blood pressure (BP) >140 mmHg, diastolic BP >90 mmHg, a rise in systolic BP >30 mmHg, or a rise in diastolic BP >15 mm on at least 2 occasions that were 6 h apart] after 20 weeks and associated with a significant proteinuria (>300 mg per 24 h); fourthly, no corresponding adequate-matched control woman, defined as detailed further on (Fig. 1).

Selection of controls

For each case subject, putative controls were women from the cohort, with no pregnancy loss, defined as: from the same ethnic origin; having had a single successful pregnancy with no antecedent of pregnancy loss; the same age (± 1 year); living in the same town and district; with the same level of education and type of occupation; having had or not had an antecedent of induced abortion; a comparable body mass index (≤ 25 kg m^{-2}, >25 kg m^{-2} and ≤ 30 kg m^{-2}, or >30 kg m^{-2}); similar tobacco smoking habits before pregnancy (never, former, current) and during pregnancy (none, current: ≤ 5 cigarettes d^{-1}, 6–10 cigarettes d^{-1}, or >10 cigarettes d^{-1}); similar patterns of oral contraception use before pregnancy (never, former, last menstrual cycle: yes, or no); similar use of folate/multivitamin supplements during the 6 months before pregnancy and during pregnancy; similar daily caffeine consumption (evaluated assuming a mean caffeine content per 100 mL of 150 mg for coffee, 60 mg for tea, 4 mg for hot cocoa, and 12 mg for soft beverages containing cola); and similar mean daily alcohol consumption (a typical weekly consumption in grams of alcohol was calculated from the data given by the patient and husband). Each putative control and her male partner were requested to sign a written informed consent form in order to create a specific plasma and DNA bank. These putative controls were then screened for all the biological

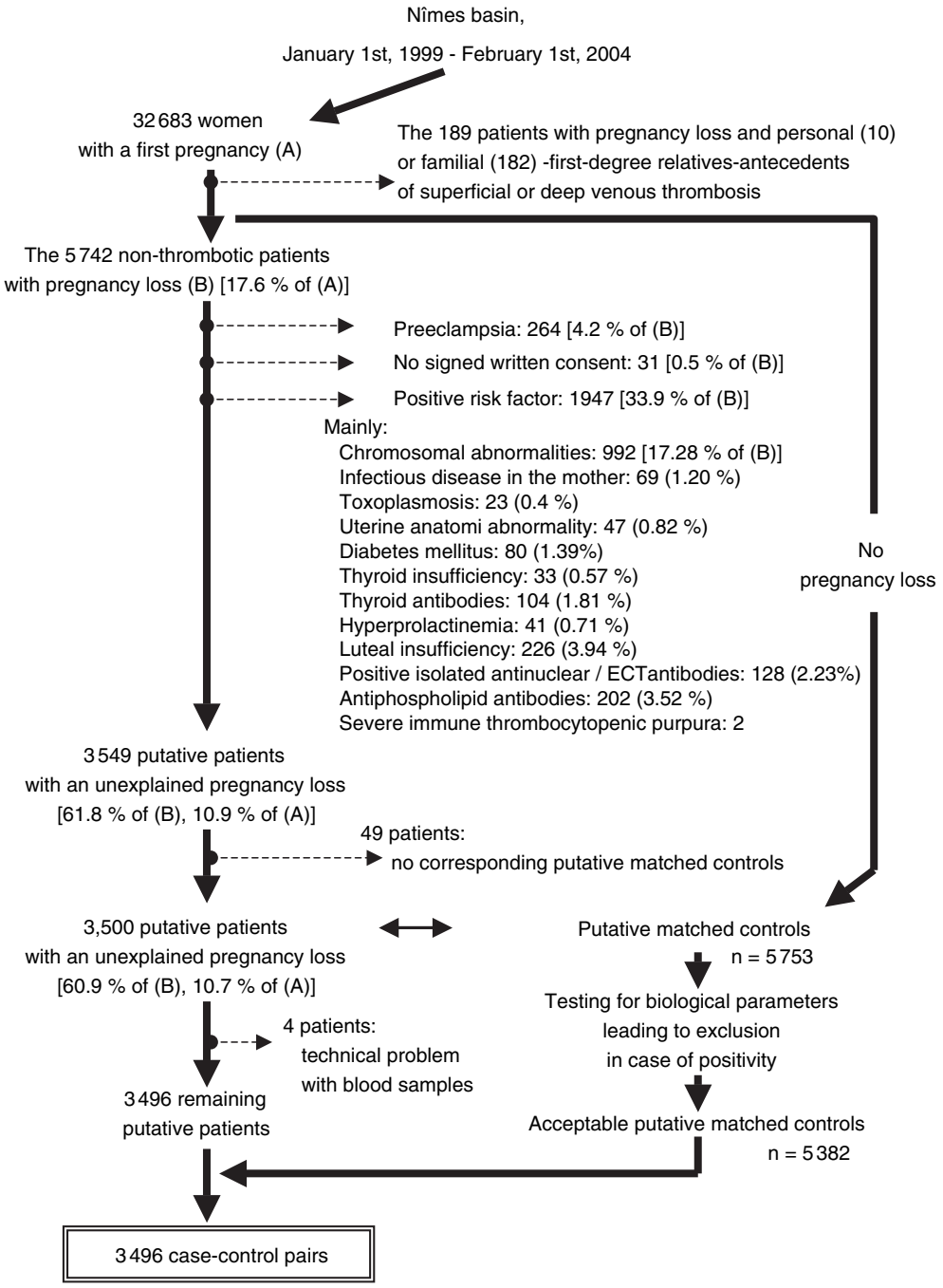

Fig. 1. The recruitment of cases and controls, the reasons for exclusion, and the composition of matched case–control pairs. Dashed lines: exclusion.

parameters, previously stated, that had led to the exclusion of patients. Individuals that successfully passed all criteria defined the group of acceptable matched putative controls. In case of multiple putative control women corresponding to a given case woman, blind random allocation was performed and matched case–control pairs were finally obtained (Fig. 1).

The clinical characteristics of the final 3496 case–control matched pairs are given in Table 1. A secondary retrospective analysis of the case–control pair characteristics was performed to look for any discordances in the matching procedure. Only 35 pairs were found with at least one discordant characteristic (Table 1). For continuous data, it was the consequence of close values belonging to two adjacent categories.

Laboratory investigation

We focused this initial work performed on the FVL and prothrombin G20210A polymorphisms and a multiplex allele-specific amplification polymerase chain reaction was used for one-step determination of FVL and FIIG20210A mutations

Table 1 Characteristics of the matched case–control pairs. no.: number

Number	3496
Age	
Median (quartiles)	28.1 (27–30)
[range]–years	[21–36]
Number of discordant pairs (±1 year):	4
Ethnic group	
White, Europe–no. (%)	2769 (79.3)
White, northern Africa–no. (%)	519 (14.8)
Black–no. (%)	126 (3.6)
Asian–no. (%)	082 (2.3)
Number of discordant pairs (categories):	0
Prepregnancy body mass index	
≤25 kg m^{-2}–no. (%)	1979 (56.6)
>25 kg m^{-2} and ≤30 kg m^{-2}–no. (%)	1238 (35.4)
>30 kg m^{-2}–no. (%)	279 (8.0)
Number of discordant pairs (categories):	6
Cigarette smoking	
Prepregnancy	
Never–no. (%)	2275 (65.1)
Former–no. (%)	685 (19.6)
Current–no. (%)	536 (15.3)
Number of discordant pairs (categories):	2
During pregnancy	
No–no. (%)	3174 (90.8)
≤5 cigarettes day^{-1}–no. (%)	211 (6.0)
>5 and ≤10–no. (%)	95 (2.7)
>10–no. (%)	16 (0.5)
Number of discordant pairs (categories):	5
Oral contraceptive use	
Never–no. (%)	1081 (30.9)
Former–no. (%)	2404 (68.8)
Last menstrual cycle: yes	1853 (53.0)
Last menstrual cycle: no	551 (15.8)
Number of discordant pairs (categories):	3
Multivitamin/folate use	
Prepregnancy–no. (%)	252 (7.2)
Pregnancy–no. (%)	672 (19.2)
Number of discordant pairs (categories):	5
Mean daily alcohol consumption (g)	
≤10–no. (%)	2253 (64.4)
>10–<50–no. (%)	1163 (33.2)
>50–no. (%)	80 (2.3)
Number of discordant pairs (categories):	5
Mean daily caffeine consumption (mg)	
< 50–no. (%)	973 (27.8)
50–< 200–no. (%)	1754 (50.2)
≥200–no. (%)	769 (22)
Number of discordant pairs (categories):	7
Antecedents of induced abortion	
Yes, vacuum aspiration–no. (%)	42 (1.2)
Number of discordant pairs (categories):	0
Level of education	
University–no. (%)	1231 (35.2)
High School–no. (%)	1824 (52.2)
College–no. (%)	441 (12.6)
Number of discordant pairs (categories):	2
Occupation	
Housewife–no. (%)	985 (28.2)
Student–no. (%)	718 (20.5)
Worker–no. (%)	1040 (29.7)
Civil servant–no. (%)	478 (13.7)
Other–no. (%)	275 (7.9)
Number of discordant pairs (categories):	6
Total number of pairs having at least one discordant characteristic	35
Only one discordance	25
Two discordances	10

[6]. Each patient's DNA was systematically analyzed with its corresponding matched control's DNA. Reference DNA, that contained each mutation as verified by direct sequence analysis, was included in each run.

Other constitutional or acquired thrombophilic conditions, like for instance clotting inhibitor deficiencies or antiphospholipid/cofactor antibodies, were not assessed.

Statistical analysis

All analyses were performed with the SPSS software, version 11 (SPSS Inc., Chicago, IL, USA). The 3496 case–control pairs were included in the statistical analysis. Genotypes were analyzed as two category variables (wild-type vs. mutated genotype) because of the absence of homozygous patients or controls in this population. Discordant and concordant pairs for FVL, then for FIIG20210AA, mutations as predictors of spontaneous unexplained pregnancy loss were analyzed to derive McNemar odds ratios (OR). This analysis was repeated in strata defined by ethnic origin (Caucasian vs. other), and timing of pregnancy loss (3–9 weeks, 10–19 weeks, 20–39 weeks, or 10–39 weeks). Conditional logistic regression was used to test whether differences in OR between strata were statistically significant (i.e. effect-modification), and to examine independent effects of the two mutations (FVL and FIIG20210AA) in multivariate analysis.

Results

Pregnancy loss during the first intended pregnancy was observed in 5931 women (189 with personal or familial venous thrombotic antecedents and 5742 without such antecedents) out of the 32 683 prospectively followed women (18.1%). After exclusion of those with pre-eclampsia and any positive risk factors, the residual putative patients with an unexplained pregnancy loss accounted for 10.9% of the initial cohort and for 59.8% of all pregnancy losses.

Globally, the FVL mutation was found in 5.32% of the included patients and 1.72% of the included controls. The prothrombin G20210A mutation was found in 3.78% of the patients and 1.60% of the controls. However, the rates of mutation positivity were heterogeneous depending on the ethnic origin of the women. Notably, mutation positivity was higher in Caucasian women than in non-Caucasians: FVL in patients (182/2769, or 6.57%, in Caucasians and 4/727, or 0.55%, in non-Caucasians, $P < 0.001$) and in controls (57/2769, or 2.06%, in Caucasians and 3/727, or 0.41% in non-Caucasians, $P = 0.001$) and FIIG20210A in patients (130/2769, or 4.70%, in Caucasians and 2/727, or 0.28% in non-Caucasians, $P < 0.001$) and in

controls (55/2769, or 1.99% in Caucasians and 1/727, or 0.14% in non-Caucasians, $P < 0.001$). Both mutations were associated with a significant risk of pregnancy loss in Caucasians [FVL: matched OR 3.19, 95% confidence interval (CI) 2.37–4.30, $P < 0.001$; FIIG20210A: OR 2.36, 95% CI 1.72–3.24, $P < 0.001$), but not in non-Caucasians (FVL: OR 1.33, 95% CI 0.30–5.96, $P = 0.71$; FIIG20210A: OR 2.00, 95% CI 0.18–22.06, $P = 0.57$). However, the differences in OR between Caucasians and non-Causasians were not statistically significant (FVL: $P = 0.26$, FIIG20210A: $P = 0.89$).

Table 2 Discordant and concordant pairs according to the presence/absence of the FVL or prothrombin G20210A (FIIG20210A) mutations, and the categories of timing of pregnancy loss

Numbers of pairs	Total	Both members negative	Both members positive	Only case positive	Only control positive
FVL					
Overall	3496	3250	0	186	60
Weeks 3–9	431	413	0	9	9
Weeks 10–19	2739	2545	0	149	45
Weeks 20–39	326	292	0	28	6
Weeks 10–39	3065	2837	0	177	51
FIIG20210A					
Overall	3496	3308	0	132	56
Weeks 3–9	431	413	0	9	9
Weeks 10–19	2739	2583	0	113	43
Weeks 20–39	326	312	0	10	4
Weeks 10–39	3065	2895	0	123	47

Table 3 Conditional logistic regression results for FVL mutation and for factor II G20210A mutation (FIIG20210A) as predictors of spontaneous unexplained pregnancy loss

	Number of pairs	OR	95% CI	*P*-value
FVL mutation				
FVL overall	3496	3.10	2.32–4.15	< 0.001
FVL by duration of amenorrhea at the moment of pregnancy loss				
3–9 weeks	431	1.00	0.40–2.52	1.00
10–39 weeks	3065	3.47	2.54–4.74	< 0.001
Difference between strata				0.012
FVL by duration of amenorrhea at the moment of pregnancy loss				
3–9 weeks (1)	431	1.00	0.40–2.52	1.00
10–19 weeks (2)	2739	3.31	2.37–4.62	< 0.001
20–39 weeks (3)	326	4.67	1.93–11.27	0.001
Difference between strata				0.035
Between 1–2				0.017
Between 2–3				0.48
Between 1–3				0.018
Factor II 20210G > A mutation				
FIIG20210A overall	3496	2.36	1.72–3.22	< 0.001
FIIG20210A by duration of amenorrhea at the moment of pregnancy loss				
3–9 weeks	431	1.00	0.40–2.52	1.00
10–39 weeks	3065	2.62	1.87–3.66	< 0.001
Difference between strata				0.055
FIIG20210A by duration of amenorrhea at the moment of pregnancy loss				
3–9 weeks (1)	431	1.00	0.40–2.52	1.00
10–19 weeks (2)	2739	2.63	1.83–3.73	< 0.001
20–39 weeks (3)	326	2.50	0.78–7.97	0.12
Difference between strata				0.16
Between 1–2				0.055
Between 2–3				0.94
Between 1–3				0.23

Table 4 Multivariate analysis (two models) for the FVL mutation and the factor II G20210A mutation (FIIG20210A) as predictors of spontaneous unexplained pregnancy loss during the first pregnancy

	OR	95% CI	*P*-value
Overall			
FVL	3.09	2.31–4.13	< 0.001
FIIG20210A	2.34	1.71–3.20	< 0.001
By duration of amenorrhea			
FVL weeks 3–9	1.00	0.40–2.52	1.00
FVL weeks 10–39	3.46	2.53–4.72	< 0.001
Difference between FVL strata			0.013
FIIG20210A weeks 3–9	1.00	0.40–2.52	1.00
FIIG20210A weeks 10–39	2.60	1.86–3.64	< 0.001
Difference between FIIG20210A strata			0.057

There were as many cases and controls who were positive for the FVL mutation or for the FIIG20210A mutation up to the 9th week of gestation. An excess of positive cases gradually appeared during the 10th and the 11th weeks and continued during the following weeks (Table 2).

Conditional logistic regression results for the two mutations as predictors of spontaneous unexplained pregnancy loss are given in Table 3. Both mutations were associated with an increased clinical risk. Taking into account the timing of pregnancy loss, those that occurred before week 10th were not significantly associated with either of the two mutations. The two polymorphisms increased the risk of miscarriage between the 10th through the 19th week and the FVL mutation also increased the risk of pregnancy loss from the 20th through the 39th week.

In the overall multivariate analysis model, FVL and FIIG20210A mutations were independent risk factors for unexplained miscarriage during the first pregnancy, with mean values of the OR two to three orders of magnitude higher with that of the FVL mutation being the highest (Table 4). The multivariate analysis that took into account categories of pregnancy loss showed that neither of the two mutations were risk factors before the 10th week, but were independent risk factors from the 10th to the 39th week.

Discussion

This is the first large-scale attempt to study, in non-thrombotic women, the association of the two commonest constitutional thrombophilic disorders, the FVL and the prothrombin G20210A mutations, with the risk of unexplained pregnancy loss during the first intended pregnancy. We found that in Caucasian women from a delimited Mediterranean area both mutations are associated with an increased risk of fetal loss which clinical symptoms occurring from the 10th week of pregnancy on. No definite conclusions could be drawn about

non-Caucasian women, in whom the frequency of thrombophilic mutations was exceedingly low.

All our patients and controls had been selected as being asymptomatic for venous thromboembolism. Other constitutional or acquired thrombophilic conditions, like clotting inhibitor deficiencies or antiphospholipid/cofactor antibodies, were not assessed. The presence of other maternal thrombophilic conditions may theoretically be a confounding factor of the risk estimate in carriers of the two polymorphism, but is unlikely to reverse the absence of significant risk associated with the two polymorphisms before 10 weeks. It can however modify the estimate of the risk from the 10th week of pregnancy on. Future studies will focus on maternal hemostatic particularities acting as protecting or enhancing risk cofactors.

The overall rate of pregnancy loss was high (18.1%) in clinically recognized pregnancies. Previous studies gave a spontaneous miscarriage rate varying around 12–15% [7–10]. Investigations in China evidenced an overall miscarriage rate of 9.1% during the first pregnancy: [11] a urine pregnancy test was performed until to 2 weeks after a missed menstrual period, whereas we used blood analysis during the first week. Recent British data gave a miscarriage rate of between 12% and 13% [12]. These differences may be due to variations in the assessment methods used, or to true differences in rates of miscarriage between these populations.

One limitation is that we cannot assess clinically unrecognized miscarriages. However, we could assess whether clinically recognized miscarriages were diagnosed at similar durations of amenorrhea, leading us to analyze the risk of pregnancy loss with regard to durations of amenorrhea from third weeks on.

There may be a difference between the moment of embryo/fetal death and the onset of clinical symptoms leading to diagnosis, but we could not perform systematic ultrasonographic evaluations. Many of the embryo/fetal deaths could have been substantially earlier than the onset of the first-related clinical symptom. This probably explains the gradual increase of risk from the 9th week to the 11th, some losses during the 10th week probably reflecting earlier deaths. However, our goal was to look for very practical landmarks, leading to imagine, if any, easy-to-apply proposals for investigations in patients. A clinical and simple definition of the pathology under focus was thus necessary.

Patients with thrombotic antecedents were not included. Some of them had been previously investigated before this study, and some received prophylactic low-molecular weight heparin during pregnancy. Including them would have biased our study.

Case–control pairs were matched for clinical parameters potentially interacting with the prognosis of pregnancy [13–22]. Antecedents of medically induced abortion were accepted, due to the absence of impact in nulliparous women on the outcome of a subsequent pregnancy [23]. Our results are unlikely to be biased by a consistent excess of classical risk factors in one of the groups of women.

The low prevalence of both mutations found in our Caucasian control group (roughly 2%) is characteristic of this population. This region was less consistently penetrated by the migrating Neolithic farmers who expanded the FVL in Europe and generated the heterogeneity of the FVL prevalence in France than other French regions. Invaders then pirated from North Africa, known for the low prevalence of the two mutations [24,25], occurred later on, with episodes of long-lasting presence: during the 8th century, Nîmes was under the domination of the Arabic empire. Lucotte and Mercier found on a limited sample of individuals from Montpellier, situated 50 km west from Nîmes, a higher prevalence of FVL [26] but Nimes basin still contains a more native population. The prevalence of the prothrombin A20210G polymorphism agrees with the overall estimate in Europe [27].

It is not until the beginning of the 8th week of pregnancy that communications between the maternal uterine spiral arteries and the placental intervillous space can be recognized [28]. Ultrasonography has shown that the placenta replaces the yolk sac as the source of blood supply to the embryo between the beginning of the 8th and the 10th weeks of gestation [29]: maternal blood hypercoagulability is unlikely to play a deleterious role before this period. As for all case–control studies, our main limitation is that the causality of the observed associations cannot be established. However, our results deal with the switch between the yolk sac and the umbilicoplacental circulations and with more fundamental results obtained in knock-out animals demonstrating that maternal hypercoagulability in the intervillous space modulates the growth and survival of trophoblast cells. [30] Therefore, the association noted by Rey *et al.* [1] Kovalevsky *et al.* [2] and Dudding and Attia [3] of first trimester losses with FVL or FIIG20210A may reflect increased pregnancy losses from the 10th week. We agree with Roqué *et al.* [4] that the first trimester should not be viewed as a homogeneous interval.

Finally, in women with a first intended pregnancy, 9.8% of the unexplained miscarriages recognized throughout the onset of clinical symptoms from the 10th week of amenorrhea were associated with the two mutations. A low-molecular weight heparin induces more successful second pregnancies than low-dose aspirin in women with a mutation [31]. We propose that women with a first pregnancy, and unexplained pregnancy loss from the 10th week, should be screened for these two mutations. The individualization of all the cofactors should help understand which couples are at very high risk. New developments from our NOHA first study are warranted.

Addendum

Drs Gris, Lissalde-Lavigne, Quéré, Dauzat and Marès designed the study. Drs Lissalde-Lavigne, Ripart-Neveu, Balducchi and Gris gathered the data. Drs Fabbro-Peray, Daurès and Perneger analyzed the data. Statistical expertise was performed by Drs Perneger. Mercier and Cochery-Nouvellon co-ordinated and performed laboratory investigations. Drs Gris and Lissalde-Lavigne drafted the manuscript. All authors critically reviewed the manuscript.

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References

1 Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet* 2003; **361**: 901–8.
2 Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss. *Arch Intern Med* 2004; **164**: 558–63.
3 Dudding TE, Attia J. The association between adverse pregnancy outcomes and maternal factor V Leiden genotype: a meta-analysis. *Thromb Haemost* 2004; **91**: 700–11.
4 Roqué H, Paidas MJ, Funai EF, Kuczynski E, Lockwood CJ. Maternal thrombophilias are not associated with early pregnancy loss. *Thromb Haemost* 2004; **91**: 290–5.
5 Gris JC, Quéré I, Sanmarco M, Boutière B, Mercier E, Amiral J, Hubert AM, Ripart-Neveu S, Hoffet M, Tailland ML, Rousseau O, Montpeyroux F, Dauzat M, Sampol J, Daurès JP, Berlan J, Marès P. Antiphospholipid and antiprotein syndromes in non-thrombotic, non-autoimmune women with unexplained recurrent primary early foetal loss. The Nîmes Obstetricians and Haematologists study[4]. *Thromb Haemost* 2000; **84**: 228–36.
6 Hézard N, Cornillet-Lefebvre P, Gillot L, Potron G, Nguyen P. Multiplex ASA PCR for a simultaneous determination of factor V Leiden gene, G > A 20210 protrombin gene and C > T 677 MTHFR gene mutations. *Thromb Haemost* 1998; **79**: 1054–5.
7 Miler JR, Williamson E, Glue J, Gordon YB, Grudzinskas JG, Sikes A. Fetal loss after implantation: a prospective study. *Lancet* 1980; **ii**: 554–6.
8 Edmonds DK, Lindsay KS, Miller JR, Williamson E, Wood PJ. Early embryonic mortality in women. *Fertil Steril* 1982; **38**: 447–53.
9 Whittaker PG, Taylor A, Lind T. Unexpected pregnancy loss in healthy women. *Lancet* 1983; **i**: 1126–7.
10 Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Amstrong EG, Nisula BC. Incidence of early loss in pregnancy. *N Engl J Med* 1988; **319**: 189–94.
11 Gindler J, Li Z, Berry RJ, Zheng JC, Correa A, Sun XM, Wong LY, Cheng LC, Erickson JD, Wang Y, Tong QL. Folic acid supplements during pregnancy and risk of miscarriage. *Lancet* 2001; **358**: 796–800.
12 Maconochie N, Doyle P, Prior S. The National Women's Health Study: assembly and description of a population-based reproductive cohort. *BMC Public Health* 2004; **4**: 35.
13 Anderson AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. *Br Med J* 2000; **320**: 1708–12.
14 Lashen H, Fear K, Sturdee DW. Obesity is associated with increased risk of first trimester and recurrent miscarriage: matched case-control study. *Hum Reprod* 2004; **19**: 1644–6.
15 Ness RB, Grisso JA, Hirschinger N, Markovis N, Shaw LM, Day NL, Kline J. Cocaine and tobacco use and the risk of spontaneous abortion. *N Engl J Med* 1999; **340**: 333–9.
16 George L, Mills JL, Johansson AL, Nordmark A, Olander B, Granath F, Cnattingins S. Plasma folate levels and risk of spontaneous abortion. *JAMA* 2002; **288**: 1867–73.
17 Signorello LB, McLaughlin JK. Maternal caffeine consumption and spontaneous abortion: a review of the epidemioloc evidence. *Epidemiology* 2004; **15**: 229–39.
18 Henriksen TB, Hjollund NH, Jensen TK, Bonde JP, Anderson AM, Kolstad H, Ernst E, Giwercunan A, Skakkebaek NE, Olsen J. Alcohol consumption at the time of conception and spontaneous abortion. *Am J Epidemiol* 2004; **160**: 661–7.
19 Ford JH, MacCormac L. Pregnancy lifestyle study: the long-term use of contraceptive pill and the risk of age-related miscarriage. *Hum Reprod* 1995; **10**: 1397–402.
20 Cattaruzza MS, Spinelli A. Spontaneous abortion in Italy: social differences and temporal trends. *Epidemiol Prev* 2000; **24**: 166–71.
21 Hemminki E, Merilainen J, Malin M, Rahkonen O, Teperi J. Mother's education and perinatal problems in Finland. *Int J Epidemiol* 1992; **21**: 720–4.
22 Sun Y, Che Y, Gao E, Olsen J, Zhou W. Induced abortion and risk of subsequent miscarriage. *Int J Epidemiol* 2003; **32**: 449–54.
23 Chen A, Yuan W, Meirik O, Wang X, Wu SZ, Zhou L, Luo L, Gao E, Cheng Y. Mifepristone-induced early abortion and outcome of subsequent wanted pregnancy. *Am J Epidemiol* 2004; **160**: 110–7.
24 Chafa O, Reghis A, Aubert A, Fisher AM. Prevalence of the FVQ506 (factor V Leiden) mutation in the normal and thrombophilic Algerian population. *Br J Haematol* 1997; **97**: 688–9.
25 Mathonnet F, Nadifi S, Serazin-Leroy V, Dakouane M, Giudicelli Y. Absence of factor V Leiden mutation and low prothrombin G20210A mutation prevalence in a healthy Moroccan population. *Thromb Haemost* 2002; **88**: 1073–4.
26 Lucotte G, Mercier G. Population genetics of factor V Leiden in Europe. *Blood Cells Mol Dis* 2001; **27**: 362–7.
27 Rosendaal FR, Dogen CJ, Zivelin A, Aruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. *Thromb Haemost* 1998; **79**: 706–8.
28 Boyd JD. Development of the human placenta with the first three months of gestation. *J Anat Lond* 1960; **94**: 297–328.
29 Makikallio K, Tekay A, Jouppila P. Yolk sac and umbilicoplacental hemodynamics during early human embryonic development. *Ultrasound Obstet Gynecol* 1999; **14**: 175–9.
30 Isermann B, Sood R, Pawlinski R, Zogg M, Kalloway S, Degen JL, Mackman N, Weiler H. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. *Nat Med* 2003; **9**: 331–7.
31 Gris JC, Mercier E, Quéré I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M, Ripart-Neveu S, Tailland ML, Dauzat M, Marès P. Low molecular weight heparin versus low-dose aspirin in women with one foetal loss and a constitutional thrombophilic disorder. *Blood* 2004; **103**: 3695–9.