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Factor V Leiden mutation in relation to fecundity and miscarriage in women with venous thrombosis

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BACKGROUND: Factor V Leiden mutation (Arg506Gln) increases the likelihood of venous thrombosis; it may also have a positive effect through facilitation of embryo implantation. This may manifest itself as a reduced time to pregnancy (increased fecundity) and fewer miscarriages in the first trimester. METHODS: From March 1999 onwards, consecutive patients with a first venous thrombosis (VT) were recruited. The first 115 female VT patients with factor V Leiden and 230 age-matched female VT patients without factor V Leiden were included. All patients, unaware of their genotype, received a structured questionnaire. RESULTS: Of the 297 (86%) women who returned the questionnaire, 220 had been pregnant at least once. Time to first pregnancy was unaffected by carrier status: 58% factor V Leiden carriers reported a pregnancy within 3 months compared to 54% non-carriers. The miscarriage proportion was 14%, similar in both groups. First trimester miscarriage was less frequent among carriers (46%) than among non-carriers (95%) (relative risk 0.5, 95% confidence interval 0.3–0.9). CONCLUSIONS: Factor V Leiden mutation may support embryo implantation, as factor V Leiden carriers had fewer miscarriages in the first trimester with a similar overall miscarriage rate. Miscarriage of embryos with poor viability may be postponed until the second trimester in factor V Leiden carriers. Fecundity was not influenced by factor V Leiden status.

Key words: Factor V Leiden/fecundity/miscarriage/time to pregnancy

Introduction

Factor V Leiden mutation (Arg506Gln) is present in 4–10% of people of Caucasian origin (Bertina *et al.*, 1994; Rees, 1996). The mutation induces a hypercoagulable state which increases the risk of venous thrombosis 7-fold among heterozygous carriers and ~80-fold among homozygous carriers compared to non-carriers (Rosendaal *et al.*, 1995). It has been suggested that factor V Leiden mutation may be associated with negative outcomes of reproduction such as (recurrent) abortion, pre-eclampsia, prematurity and small-for-gestational-age neonates. However, the available data are conflicting (Rai *et al.*, 2001; Morrison *et al.*, 2002; Hundsdoerfer *et al.*, 2003; Pauer *et al.*, 2003). Because of the high prevalence of this mutation in certain populations, positive effects associated with factor V Leiden have been postulated, possibly through human reproduction. Women who carry the factor V Leiden mutation lose less blood in menstruation, have higher haemoglobin levels, and possibly a lower incidence of life threatening post-partum haemorrhage, which could be an evolutionary advantage (Lindqvist *et al.*, 2001). Furthermore, a higher than expected prevalence of factor V Leiden mutation carriers was found in healthy pregnant women (9.2%) (De Groot *et al.*, 1999) compared to the general population (3%) (Rosendaal *et al.*, 1995). A similar finding was reported in a recurrent miscarriage study where the prevalence of factor V Leiden was higher in women without a history of recurrent miscarriages (14%) compared to those with recurrent miscarriages (1.7%) (Dilley *et al.*, 2002). Facilitation of embryo implantation was suggested as a possible positive pathway for factor V Leiden (Majerus, 1994). In agreement with this hypothesis, an improved implantation rate in factor V Leiden carriers compared to non-carriers was reported in ICSI pregnancies. If either the mother or the fetus carried the factor V Leiden mutation, the proportion of live births was 90% (9/10) for the first embryo transfer compared to 49% (45/92) in factor V Leiden negative pairs (Göpel *et al.*, 2001). A reduced time to pregnancy (increased fecundity) in spontaneous pregnancies of factor V Leiden carriers would be an indication of a protective effect of factor V Leiden.

To study the effect of factor V Leiden mutation on embryo implantation and human reproduction, we investigated

the association of factor V Leiden mutation on fecundity and miscarriages and the trimester in which the miscarriages occurred in 297 women. To be able to construct a large cohort of women with factor V Leiden, we used information from a large study on venous thrombosis. We also investigated the effect of factor V Leiden carriership on term birth rate and birthweight.

Materials and methods

The women described in this study were enrolled in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study (MEGA study). The primary aim of the MEGA study is to assess interaction between environmental and genetic risk factors for venous thrombosis. From March 1999 onwards, all consecutive patients who suffered a first deep-vein thrombosis or pulmonary embolism between the age of 18 and 70 years were recruited from six anticoagulation clinics in The Netherlands. The anticoagulation clinics monitor the anticoagulant therapy of all patients in a well-defined geographical area, which allowed us to identify consecutive and unselected patients with venous thrombosis. All participants filled in a questionnaire on risk factors for venous thrombosis. A blood sample was drawn 3 months after discontinuation of anticoagulation therapy. Subjects who were unable or declined to give a blood sample were given the opportunity to give DNA by use of a buccal swab. All participants completed an informed consent form. The Leiden University Medical Center medical ethics committee approved the study.

For the present analysis, the first 115 female thrombosis patients identified with factor V Leiden mutation were matched by age to 230 female thrombosis patients (controls). Besides the age matching and absence of factor V Leiden, controls were randomly selected from the study participants. All 345 patients, who were unaware of their genotype, were asked to fill in an additional structured questionnaire concerning their reproductive history. Questions consisted of age at first pregnancy attempt, the period of unprotected intercourse until the desired pregnancy occurred, number of pregnancies, and the duration of each pregnancy. When there had been no pregnancies, we enquired whether this was despite efforts to become pregnant (infertility). If there was no response to the initial questionnaire, a written reminder accompanied with an identical questionnaire was sent after 3 weeks. To increase response, patients were contacted by telephone if they did not return the questionnaire after an additional 5 weeks.

Time to pregnancy was defined as self-reported time between unprotected intercourse and the occurrence of pregnancy. Miscarriage ratio was calculated as the total number of miscarriages per number of pregnancies. First trimester miscarriage was defined as embryonic or fetal loss before the completion of 12 weeks gestation; second trimester miscarriage was defined as fetal loss from 13 to 24 weeks gestation; stillbirth was defined as a loss after 24 weeks gestation. A number of factors known to influence miscarriage or fecundity were included in the questionnaire, such as age at first pregnancy attempt, age at first birth, level of education (primary school, secondary school, college or university), smoking habits, and alcohol use. The body mass index [BMI: weight in kg/(height in m)^2] was calculated at the time of thrombosis.

DNA was isolated from whole blood or buccal swabs. For the latter, three large cotton swabs in a total of 6 ml sodium dodecyl sulphate–proteinase K solution (100 mmol/l NaCl, 10 mmol/l EDTA, 10 mmol/l Tris–HCl pH 8.0, 0.5% SDS, 0.1 mg/ml proteinase K) were obtained from each patient. Upon arrival, the proteinase K concentration was raised to 0.2 mg/ml and the sample was incubated for 2 h at 65°C. Subsequently, the suspension was recovered by centrifugation. Potassium acetate was added to a final concentration of 1.6 mol/l. After 15 min incubation on ice, proteins were removed using chloroform–isoamylalcohol (24:1) treatment. The DNA in the water phase was subsequently ethanol-precipitated. After centrifugation, the pellet was resuspended in 200 ml 10 mmol/l Tris–HCl, 10 mmol/l EDTA pH 8.0 and frozen at −20°C until further analysis. Assessment of the factor V Leiden mutation in DNA retrieved from the buccal swabs was performed identically to the method for DNA from whole blood, and determined by PCR and *Mnl I* restriction digestion as described elsewhere (Bertina *et al.*, 1994).

Data are presented as simple counts and percentages. Relative risks (RR) were computed as the ratio of these counts, and 95% confidence intervals (CI) were based on a binomial distribution.

Results

A total of 297 (86%) women returned a completed questionnaire: 89% of the factor V Leiden carriers and 84% of the non-carriers. Of the 48 non-responders, four women were deceased, six were lost to follow-up, and for 21 women the reason for not returning the questionnaire was unknown. Seventeen questionnaires were returned blank because of: lack of motivation (four women), three women were too ill, for two women it brought back too many painful memories (both non-carriers), and in eight cases no reasons were given.

Of the 102 factor V Leiden carriers who returned the completed questionnaire, 80 (78%) had been pregnant at least once compared to 140 (72%) of the 195 non-carriers (RR 1.1, 95% CI 0.95–1.2). Patient characteristics of these 220 women are listed in Table I. The reasons for not having had a pregnancy were similar; infertility was reported by 5% (1/22) of factor V Leiden carriers and 11% (6/55) of non-carriers (RR 0.4, 95% CI 0.1–3.3).

Time to first pregnancy was similar for factor V Leiden carriers and non-carriers. In both groups, >90% of those

Table I. Patient characteristics and reproductive outcome of the 220 women who had been pregnant, according to factor V Leiden carrier status

	Factor V Leiden carrier[a] ($n = 80$)	Factor V Leiden non-carrier ($n = 140$)
Age at thrombosis (years)	45.1 (20–68)	44.4 (20–70)
Age at questionnaire (years)	47.6 (23–71)	46.8 (23–72)
Menstrual cycle, regular	66 (84)	118 (84)
Education, college/university	32 (45)	52 (43)
Smoked, ever	50 (63)	77 (55)
Body mass index (kg/m^2)	27 (18–58)	27 (17–57)
Age at first pregnancy attempt (years)	24.1 (15–36)	24.1 (15–38)
Age at birth first child (years)	25.2 (16–36)	25.3 (15–39)
No. of pregnancies	2.2	2.7
No. of liveborn children	1.7	1.9
Total no. of miscarriages	25 (14)	50 (14)
Stillbirths	2 (1)	9 (2)
Ectopic pregnancies	0 (−)	4 (1)
Planned abortion	10 (6)	17 (4)
Other (e.g. twins)	4 (2)	9 (2)

Values are mean (range) or *n* (%).
[a]One homozygous factor V Leiden mutation carrier.

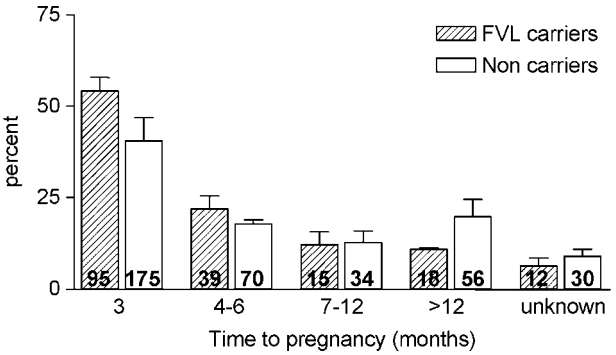

Figure 1. Fecundity (time to pregnancy) for all pregnancies in 220 venous thrombosis patients according to factor V Leiden mutation (179 pregnancies in factor V Leiden carriers and 365 pregnancies in non-carriers, number of pregnancies stated in the bars).

who had been pregnant could recall the time to their first pregnancy. Forty-two (58%) factor V Leiden carriers achieved their first pregnancy within 3 months compared to 70 (54%) of the non-carriers (RR 1.1, 95% CI 0.8–1.4). Nine (13%) factor V Leiden carriers reported a time to first pregnancy of >12 months, which had occurred in 21 (16%) of the non-carriers (RR 0.8, 95% CI 0.4–1.6). When all consecutive pregnancies per woman were combined, similar results were found (Figure 1).

The miscarriage proportion for all pregnancies was similar in both groups, 14% (25/179) for factor V Leiden carriers and 14% (50/365) for non-carriers (RR 1.0, 95% CI 0.6–1.6). Considering only the first pregnancy, 16% (13/80) of the factor V Leiden carriers had experienced a miscarriage compared to 15% (21/140) of the non-carriers (RR 1.1, 95% CI 0.6–2.0). However, the trimester in which the miscarriages occurred was different according to factor V Leiden status; factor V Leiden carriers who experienced a miscarriage during their first pregnancy had fewer miscarriages in the first trimester compared to non-carriers, respectively 46 and 95% (RR 0.5, 95% CI: 0.3–0.9). Subsequently, factor V Leiden carriers had more miscarriages in the second trimester (RR 10.8, 95% CI 1.5–77.7) (Table II). When all consecutive pregnancies per woman were combined, the difference persisted (Table II). Stillbirth (fetal loss in the third trimester) was rare, and was equal in both groups. For the 80 first pregnancies of factor V Leiden carriers, no stillbirths occurred compared to four (3%) stillbirths in 140 first pregnancies in non-carriers. For all pregnancies per woman combined, two (1%) stillbirths occurred in 179 pregnancies in factor V Leiden carriers compared to nine (2%) in 365 pregnancies in non-carriers (RR 0.4, 95% CI 0.1–2.1).

The proportion of live births was similar in both groups; 74% (59/80) among factor V Leiden carriers compared to 76% (106/140) among non-carriers (RR 1.0, 95% CI 0.8–1.1). Term birth (37–42 weeks gestation) in first pregnancies was comparable, 64% (38/59) and 69% (73/106) respectively (RR 0.9, 95% CI 0.7–1.2). Mean birthweight for the children born at term was similar for factor V Leiden carriers (3644 g, range 2500–4000) and non-carriers (3481 g, range 1200–3975).

Table II. Miscarriages, per trimester, out of 220 women who had been pregnant, according to factor V Leiden (FVL) mutation

	First trimester (≤12 weeks)	Second trimester (13–24 weeks)	Total
First pregnancy only (80 pregnancies in FVL carriers and 140 in non-carriers)			
FVL+	6 (46)	7 (54)	13
FVL−	19 (95)	1 (5)	20[a]
Relative risk (95% CI)	0.5 (0.3–0.9)	10.8 (1.5–77.7)	
All pregnancies combined (179 in FVL carriers and 365 in non-carriers)			
FVL+	16 (64)	9 (36)	25
FVL−	43 (90)	5 (10)	48[b]
Relative risk (95% CI)	0.7 (0.5–1.0)	3.5 (1.3–9.2)	

Values are *n* (%).
[a]Of one non-carrier, the trimester in which the miscarriage took place was unknown.
[b]Of two non-carriers, the trimester in which the miscarriage took place was unknown.
CI = confidence interval.

Discussion

In this study of 297 women with venous thrombosis, we found no association between factor V Leiden mutation and fecundity or the frequency of miscarriage. However, when miscarriages had occurred, they took place less often in the first trimester in factor V Leiden mutation carriers than in non-carriers.

Miscarriages occurred as often in factor V Leiden carriers (14%) and non-carriers (14%), in percentages that are similar to the general population (10–15%) (Zinaman *et al.*, 1996). Published data on factor V Leiden in relation to miscarriages are conflicting. Recent meta-analyses have shown an association between factor V Leiden and recurrent fetal loss, occurring both in early and in late in gestation (respectively OR 2.01, 95% CI 1.13–3.58 and OR 7.83, 95% CI 2.83–21.67) (Rey *et al.*, 2003; Kovalevsky *et al.*, 2004). For non-recurrent early loss (<19 weeks gestation) no clear association was found with factor V Leiden (OR 1.40, 0.66–2.97); and for non-recurrent isolated second/third trimester loss (stillbirth >19 weeks) a positive association was found (OR 3.26, 95% CI 1.82–5.83) (Rey *et al.*, 2003; Dudding and Attia, 2004).

The focus in the literature has mainly been on factor V Leiden in relation to second and third trimester loss, as thrombosis of the placental vessels is assumed to be an important factor in fetal loss. In our study, factor V Leiden carriers experienced more fetal loss in the second trimester, as would be expected by the placental vessel thrombosis theory. A clear increase in third trimester loss would also be expected, with an even higher rate of fetal loss in third than in second trimester for factor V Leiden carriers. However, third trimester loss (stillbirth) was equally distributed over carriers and non-carriers, with a similarly low rate, which contradicts this theory. Moreover, in the present study, mean birthweight was not influenced by factor V Leiden status, which is in line with earlier published data (Lindqvist *et al.*, 1999). If we consider hypercoagulability to lead to obstruction of placental vessels as a major pathological factor

among pregnant factor V Leiden carriers, one would not expect an effect on first trimester miscarriages, since the placental circulation in the first trimester has not yet been fully established (Hustin and Schaaps, 1987; Burton *et al.*, 1999). Therefore, impaired placental perfusion might not be critical for the embryonic development and implantation during (very) early gestation. We cannot differentiate between the types of early pregnancy loss in this study (e.g. presence or absence of fetal heart prior to the loss), as all the data were provided to us by the patients through interview. Furthermore, most would not have had an early first trimester scan, as they are not routinely done in The Netherlands.

Our results indicate a clear reduction in first trimester loss in factor V Leiden carriers, which is subsequently compensated by an increased loss in the second trimester. Similar findings were reported in a recent study where a decreased risk of recurrent pregnancy losses at <10 weeks gestation was found in factor V Leiden carriers (OR 0.23, 95% CI 0.07–0.77) with a subsequent increase in losses after 10 weeks (Roqué *et al.*, 2004). This suggests a protective effect on the embryo during the first trimester in factor V Leiden carriers, including less viable embryos that will eventually abort in the second trimester. This may explain the similar overall frequency of miscarriages in factor V Leiden carriers and non-carriers. A lower frequency of miscarriages in the first trimester may thus reflect a successful implantation. Approximately 50–70% of all miscarriages are attributable to detectable chromosomal abnormalities, furthermore 15–20% is thought to be due to morphological defect(s) in the embryo. A recent study confirmed this with a transcervical embryoscopy at the time of the curette and cytogenetic analysis of the products of conception (Philipp *et al.*, 2003). We have no cytogenetic information in our study, as karyotyping of the products of conception is not a routine consideration in The Netherlands. The possible protective effect of factor V Leiden early in pregnancy will require further study with, among other things, cytogenetic testing of the miscarriage products and possibly transcervical embryoscopy prior to evacuation.

Our study did not show a clearly increased fecundity in factor V Leiden carriers. Many factors affect fecundity, including physiological, behavioural and environmental factors. Known factors, such as age at pregnancy attempt, regularity of the menstrual cycle, smoking habits and educational level were similar in factor V Leiden carriers and non-carriers. As fecundity is a manifestation of both conception and implantation, it is important to further distinguish these. For conception, factors such as sperm quality, coital frequency and timing are of great importance. However, this study was not designed to examine these factors and it remains unclear whether factor V Leiden has any effect on these factors. Aspects influencing implantation have not yet been fully elucidated; however, improved implantation could be due to an increase in the hypercoagulable state, related to the factor V Leiden mutation. This was suggested by a study that omitted conception by reporting only on ICSI pregnancies, reflecting implantation success (Göpel *et al.*, 2001). A higher incidence of implantation success was found if either the mother or the fetus was a factor V Leiden carrier. It is possible that the beneficial effect of factor V Leiden on implantation alone has a less clear affect on fecundity compared to various factors concerning conception, and therefore, in our study, fails to show an overall difference in fecundity.

We investigated the reproductive histories of women who had suffered venous thrombosis. The choice of this design was opportunistic, in that it offered the opportunity to study a large cohort of factor V Leiden carriers. We have considered whether this choice, rather than the ideal study of factor V Leiden carriers without thrombosis, could have distorted our results. First, the period about which questions were asked preceded the thrombotic event, in most cases by many years. Hence, the thrombotic event itself did not influence our results. As the patients developed thrombosis, they will have had more risk factors for thrombosis than other women. It is known that this is not only true for women without factor V Leiden but also for women with factor V Leiden (the majority of people with factor V Leiden never develop thrombosis, and there must be causes why some do). For this reason, we chose thrombosis cases without factor V Leiden as controls rather than healthy women without factor V Leiden, and therefore differences between the group can be attributed to factor V Leiden. One could argue that the groups differed more: women with thrombosis without factor V Leiden probably had more additional risk factors than those with factor V Leiden, for instance another, possibly still unknown, gene defect. This could, if that other prothrombotic factor also affected implantation and fetal loss, explain the absence of a difference between the groups in the frequency of miscarriage. However, we did find a difference.

As the reproductive data in the present study were collected by interview, recall bias is possible, but seems unlikely. Validation studies of fecundity and miscarriages have shown a good match between long-term recall of personal reproductive history through interview and medical data (Joffe *et al.*, 1993). Moreover, the women were unaware of their factor V Leiden status at the time of the questionnaire.

In conclusion, factor V Leiden mutation may support embryo implantation, as factor V Leiden carriers reported significantly fewer miscarriages in the first trimester. This was not reflected in an increased fecundity. The overall miscarriage proportion was not influenced by factor V Leiden status. These results suggest that factor V Leiden offers a protective effect on early pregnancy and that the miscarriage of embryos with poor viability in factor V Leiden carriers is postponed until the second trimester.

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