Genetic factors in human reproduction a trade off between procreation and longevity

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Gender-specific association of the factor V Leiden mutation with fertility and fecundity in a historic cohort. The Leiden 85-Plus Study

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ABSTRACT

BACKGROUND: Factor V Leiden (FVL, Arg506Gln) mutation may facilitate embryo implantation and increase fertility and fecundity. This was studied in subjects who were of childbearing age in a time with minimal fertility control without modern contraceptive methods.

METHODS: From 1986 to 1999, 1502 inhabitants of Leiden, The Netherlands, reaching the age of 85 years were enrolled in the Leiden 85-Plus Study. Of 1176 subjects the FVL status was analysed, in 365 male and 811 female subjects.

RESULTS: The FVL carrier rate was 4.3%. Fertility was not affected by FVL status. In male subjects, fecundity (interval between marriage and birth of first child) was significantly increased in FVL carriers; 67% of male FVL carriers had a child within 371 days of marriage (therefore conceived within 3 months of marriage), compared with 19% of male non-carriers [relative risk (RR), 3.5; 95% confidence interval (CI), 2.1–5.7; $P < 0.001$]. Within 6 months of marriage, 75% of male FVL carriers had conceived a child compared with 34% male non-carriers (RR, 2.2; 95% CI, 1.5–3.2; $P = 0.01$). In female subjects, fecundity was not influenced by FVL status.

CONCLUSION: Fecundity is increased in male FVL carriers; in female subjects, no such association was observed.

Factor V Leiden (FVL, Arg506Gln) mutation is present in 4–10% of people of Caucasian origin (Bertina *et al*., 1994; Rees, 1996). The FVL mutation induces a hypercoagulable state which increases the risk of venous thrombosis three- to sevenfold among heterozygous carriers and about eightfold among homozygous carriers compared to non-carriers (Rosendaal *et al*., 1995).

The persistence and high prevalence of the FVL mutation in the general population suggests that it may carry an evolutionary advantage. As early as 1957, George Williams proposed the 'antagonistic pleiotropy' theory (Williams, 1957). Briefly, this theory states that ageing is due to the decline of the force of natural selection late in life and that the fixation of alleles with positive effects upon fitness early in life also have deleterious effects late in life. This 'antagonistic pleiotropy' theory may apply to FVL since a positive effect on implantation has been suggested (Majerus, 1994). This positive effect was subsequently verified in a study where an improved implantation rate in ICSI pregnancies was reported if either the mother or the child carried the FVL mutation (Göpel *et al*., 2001).

Some evolutionary benefit of FVL mutation in females may lie in the fact that women who carry the FVL mutation lose less blood in menstruation, have higher haemoglobin levels and possibly have a lower incidence of life-threatening post-partum haemorrhage (Lindqvist *et al*., 2001). On the other hand, FVL mutation in females might also be associated with negative outcomes of reproduction such as recurrent abortion, pre-eclampsia, prematurity and small-for-gestational-age neonates (De Groot *et al*., 1999; Rai *et al*., 2001; Morrison *et al*., 2002; Hundsdoerfer *et al*., 2003; Pauer *et al*., 2003; Krabbendam *et al*., 2005). As the inheritance pattern of FVL can best be described as co-dominant, the status of both maternal and paternal FVL is likely to be of significance. FVL status in males in relation to reproduction has not been investigated to date. Although it seems unlikely that FVL status *per se* would influence male fertility, no published data on this topic are available. Whether the FVL status of the embryo as such has any influence on reproductive success remains to be clarified.

A high fecundity rate (shorter time to a desired pregnancy) may reflect implantation success. However, in a recent study concerning only females that had suffered venous thrombosis, FVL was not associated with a change in fecundity (van Dunné *et al*., 2005). A population of males and females with their fertile years in an era in which fertility control was minimal

appears suitable for the analysis of the influence of FVL on fertility and fecundity. In this study, we assess fertility and fecundity in a large cohort of subjects born in the late nineteenth and early twentieth centuries, who were of childbearing age in a time where modern contraceptive methods were unavailable.

MATERIALS AND METHODS

The Leiden 85-Plus Study consists of two separate cohorts. A detailed description of both cohorts has been presented elsewhere (Van Aken *et al*., 2002). In short, subjects of the first cohort were enrolled between December 1986 and March 1989. During that period, a total of 977 inhabitants of Leiden, The Netherlands, who were aged 85 and over were included. A second cohort of 85-year-olds, consisting of 599 subjects, was enrolled between September 1997 and September 1999. There were no selection criteria for health or demographics in either cohort. Of all subjects, a blood sample was obtained. DNA was available for an unselected sample of 660 subjects in the first cohort (68%) where the FVL mutation could be determined in 653 subjects. In the second cohort, cell material was available for 561 subjects (94%) where the FVL mutation could be determined in 555 subjects. The Registry of Births, Deaths, and Marriages of the municipality of Leiden and the Central Bureau of Genealogy (CBG), The Netherlands, provided the date of birth, date of marriage(s) and birth dates of children of all study participants. The CBG is the major documentation and information centre for family history and heraldry in the Netherlands. For 32 subjects, there was insufficient information available on their marital history or their number or dates of birth of progeny. Hence, complete information was available for 1176 subjects. The Leiden University Medical Centre's medical ethical committee approved the protocol for both cohorts.
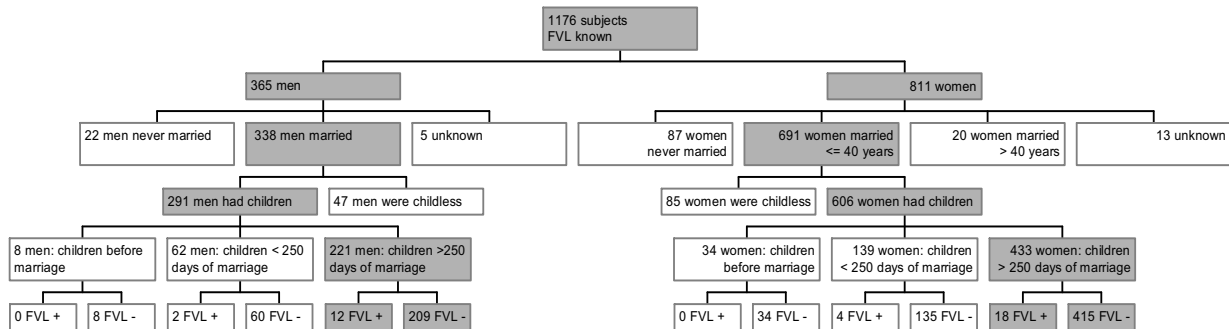
Figure 1. Selection of subjects for the Leiden 85-Plus Study with known factor V Leiden (FVL) status.

Fecundity was defined as the calculated time interval between the date of (first) marriage and the date of birth of the firstborn child. This concept of delay from marriage to the first birth has previously been defined as 'effective fecundability' (Leridon, 1977). This effective fecundability was arbitrarily divided into groups according to probable conception time. If the conception had taken place within the first 3 months of marriage, it can be assumed that these children were most likely born within 250 and 371 days of the marriage date. This was calculated by adding 3 months (91 days) to the median duration of a term pregnancy (280 days). Likewise, if conception had occurred within 6 months of marriage, the children would most likely be born between 250 and 463 days after marriage. For conception within 12 months of marriage, the date of birth was assumed to be within 250 and 645 days of marriage. To minimize the selection of pregnancies conceived before marriage, children born before marriage or within the first 36 weeks (250 days) of marriage were excluded from analysis. Women with an age beyond 40 at the time of their marriage were excluded from further analysis due to the rapid decline of fertility and fecundity that can be expected from that age onwards. Figure 1 illustrates the flow chart of the participating subjects.

For the present study, the FVL analysis was done at the same moment in time for all available 1176 stored blood samples. Assessment of the FVL mutation in DNA was determined by PCR and *Mnl*I restriction digestion as described elsewhere (Bertina *et al*., 1994; Heijmans *et al*., 1998).

The two groups were compared using Student's *t*-test for continuous variables and Pearson's chi-square test for categorical variables with Fisher's exact test applied when the expected frequencies were <5. All tests were two-tailed.

Table I. Characteristics of 338 married male and 691 female subjects married at or before 40 years of age according to their factor V Leiden carrier status.

	Men		Women	
	FVL +	FVL -	FVL +	FVL -
	(n =15)	(n =323)	(n =26)	(n =665)
Age at marriage	26 (22-39)	26 (18-61)	25.5 (19-39)	24 (17-40)
Age at birth first-born	27.5 (23-48)	28 (17-56)	27.5 (20-40)	26 (13-40)
Year of birth first-born	1935 ('21-'61)	1936 ('12-'59)	1935 ('18-'53)	1931 ('10-'54)
Number of children	2 (1-5)	3 (1-12)	2 (1-8)	3 (1-11)
Childless (%)	2 (13)	47 (15)	4 (15)	82 (12)

Values are median (range) or n (%). FVL = Factor V Leiden.

RESULTS

The 1176 subjects included in this analysis comprised 365 men (31%) and 811 women (69%). The year of birth ranged from 1883 to 1914. The FVL mutation was present in 18 men (4.9%) and 33 women (4.1%). All were heterozygous for FVL. There were no homozygotes. A total of 338 (93%) of the male subjects had been married at least once and 691 (85%) of the female subjects were married at or before the age of 40. Two hundred and ninety-one of the married men had children, 8 (3%) men had a child before marriage (all non-carriers) and 62 (21%) men had their firstborn child within 250 days of marriage and were therefore excluded (two were FVL carriers). Six hundred and six of the women married at or before the age of 40 had children, 34 (6%) had a child before marriage (all non-carriers). In 139 (23%) women the firstborn was recorded within the first 250 days of marriage (four FVL carriers) and therefore excluded under the assumption that conception had taken place before marriage. The year of birth of the firstborn child ranged from 1912 to 1961 in male subjects and from 1910 to 1954

in female subjects. The number of children was unrelated to the presence of the FVL mutation (Table I). A similar number of marriages remained childless in FVL carriers and non-carriers regardless of gender (Table I).

Table II. Assumed conception time calculated for the births occurring more than 250 days after marriage for the 221 married men (12 FVL+ and 209 FVL–) and 433 women (18 FVL+ and 415 FVL–) married at or before 40 years old

	Men		
	FVL + (%)	FVL – (%)	Relative risk (95% CI)
Conception ≤ 3 months of marriage	8 (67)	40 (19)	3.5 (2.1-5.7)
Conception > 3 months of marriage	4 (33)	169 (81)	
Conception ≤ 6 months of marriage	9 (75)	72 (34)	2.2 (1.5-3.2)
Conception > 6 months of marriage	3 (25)	137 (66)	
Conception ≤ 12 months of marriage	9 (75)	108 (52)	1.5 (1.0-2.1)
Conception > 12 months of marriage	3 (25)	101 (48)	
	Women		
	FVL + (%)	FVL – (%)	Relative risk (95% CI)
Conception ≤ 3 months of marriage	4 (22)	112 (27)	0.8 (0.3-2.0)
Conception > 3 months of marriage	14 (78)	303 (73)	
Conception ≤ 6 months of marriage	7 (39)	171 (41)	0.9 (0.5-1.7)
Conception > 6 months of marriage	11 (61)	244 (59)	
Conception ≤ 12 months of marriage	10 (56)	233 (56)	1.0 (0.6-1.5)
Conception > 12 months of marriage	8 (44)	182 (44)	

CI: confidence interval; Values are n (%).
Conception ≤3 months: birth of firstborn child within 250 and 371 days of marriage;
Conception ≤6 months: birth of firstborn child within 250 and 463 days of marriage;
Conception ≤12 months: birth of firstborn child within 250 and 645 days of marriage.

Table II presents the assumed conception time of married men and women dependent on their FVL status. In female subjects, FVL carriers had similar fecundity rates compared with non-

carriers [relative risk (RR), 0.8; 95% confidence interval (CI), 0.3–2.0; $P = 0.79$]. Male FVL carriers had a 3.5-fold (95% CI, 2.1–5.7; $P < 0.001$) increase in the probability of conception of a child within the first 3 months of marriage compared to non-carriers. Within 6 months of marriage, the results remained similar (RR, 2.2; 95% CI, 1.5–3.2; $P = 0.01$). In an additional analysis, with all births from the first day of marriage onwards included, without the 250-day threshold, the results for males remained significant (RR, 1.9; 95% CI, 1.3–2.8; $P = 0.01$ at 3 months and RR, 1.6; 95% CI, 1.2–2.2; $P = 0.03$) at 6 months.

DISCUSSION

In the present study of 1029 married male and female subjects born between 1883 and 1914, fecundity in females was unrelated to FVL status. In males, there was an unexpected, but highly statistically significant finding of an increased fecundity (shorter time period between marriage and firstborn child) in FVL carriers compared with non-carriers. There was no association between FVL mutation and fertility or family size.

Heterozygous FVL mutation was found in 4.3% of subjects, similar in male and females. This is comparable with earlier published data on FVL prevalence in the Dutch population (Rees *et al.*, 1995). There were no individuals homozygous for factor V Leiden, which is within expected numbers as the population prevalence is 0.1%.

Fecundity in females was comparable in FVL carriers and non-carriers. The current study only comprised completed pregnancies; there was no information available on pregnancies ending in miscarriage or fetal loss. Female FVL carriers may have had higher rates of miscarriages or fetal loss, reducing the amount of children born within the first year and lowering fecundity rates masking an effect of FVL on embryo implantation in females. This seems unlikely, however, as an earlier study found that the number of reported miscarriages was similar in FVL carriers compared with non-carriers (van Dunné *et al.*, 2005).

In male subjects, FVL carriers had a significantly increased fecundity compared with non-carriers. An explanation for these findings in the male population can only be speculative. The results may be real, due to a chance finding or due to a selection bias. In selecting only the

births from 250 days after marriage (assumed to be conceived after the marriage date), a bias may occur in selecting the less-fertile couples (Sallmen *et al*., 2005). The couples that get married due to an unintended pregnancy will have their babies with a shorter interval after marriage, they will be excluded and presumably, they are the most fertile. However, FVL carriers were evenly distributed in subjects with births that occurred before the first 250 days of marriage and beyond that time in both males and females. Moreover, with all the births from the first day of marriage included, the results remained similar. Although elderly subjects (over 85 years of age) were selected, the FVL prevalence has been reported to remain stable at this age, and it does not affect population mortality (Heijmans *et al*., 1998). Hypothetically, the location of the *FVL* gene could be in the proximity of an unknown, male-fertility gene elevating the risk of a mutation in that gene, resulting in an increase in sperm numbers or motility. An analogous phenomenon is seen in cystic fibrosis (CF) where mutations in *CF* genes cause typical CF symptoms but also cause congenital bilateral absence of the vas deference and infertility in 99% of males with CF (Lissens and Liebaers, 1997). Whether FVL has any effect on sperm quality or quantity has never been investigated. Furthermore, FVL may have a positive effect on implantation (Majerus, 1994) by way of the inheritance of the paternal FVL mutation by the embryo. An FVL-positive embryo may have a higher likelihood of implantation in an FVL-negative mother. Indeed, a few small studies have reported a higher-than-expected FVL mutation rate in infants born to mothers in various (normal) control groups compared with the reported prevalence of FVL mutation in the normal population (Currie *et al*., 2002; Schlembach *et al*., 2003). Further research is required to distinguish whether not only maternal FVL status but also paternal status and subsequently the embryo is of significance for reproductive success.

In the present study, with births ranging from 1918 to 1954, 25% of subjects had a child within the first year of marriage; therefore their calculated conception time was within the first 3 months of marriage. Fifty-five percentage of subjects had their first child within 21 months of marriage, corresponding with a calculated conception time within the first 12 months of marriage. In recent times, conception rates are reported considerably higher. From 1961 to 1993, fecundity rates in Britain increased significantly for both men and women. Cumulative pregnancy rates for 1961 were reported as 56% at 3 months and 79% at 12 months. For 1993, these figures were 66% and 90%, respectively (Joffe, 2000). In recent

prospective studies, clinical pregnancy rates were as high as 65–70% in the first three cycles and 81–90% in the first six cycles (Gnoth *et al*., 2003; Wang *et al*., 2003). An explanation for this increase in fecundity may be a change in general behaviour due to more knowledge about fertility and therefore a more optimal timing of intercourse. The readily available and reliable contraception nowadays will enhance family planning with an increased focus to having a child at a specific time. Furthermore, the recent prospective studies include pregnancies ending in a miscarriage, which was not available in the present study.

The current study has some limitations. All reproductive information was acquired from registries; therefore, all conception times and fecundity rates were calculated. It is possible that not all pregnancies ending in a death of the fetus at term were reported. Whether FVL carriers may have had more premature births ending in neonatal deaths remains speculative. The selected cohort was set in a time represented by minimal fertility control and no modern contraceptive methods. We have assumed that starting a family as soon as a marriage was celebrated was desired. The circumstance of the subjects at the time of their marriage is unknown. Any significant illnesses or availability of either partner in the first year after marriage is unknown. Other factors interfering with fecundity such as sperm count, regularity of menstrual cycle and frequency of intercourse are unknown. However, it is not likely that FVL itself will interfere with these factors. Male FVL carriers were not younger at marriage, and their spouses had a similar age at marriage (median 25 years old, range 22–37) to the females included in our cohort.

In conclusion, we found that the FVL mutation increases fecundity in males, with a shorter interval between marriage and birth of the first child in an era prior to modern contraceptive use. This was not found in females. Possible explanations are that FVL increases male fertility, linking the *FVL* gene to a fertility gene that may potentially increase sperm count or motility. Another explanation might be that the presence of the FVL mutation in an embryo increases the implantation rate in an FVL-negative mother. The 'antagonistic pleiotropy' theory (Williams, 1957) regarding the fixation of alleles with positive effects upon fitness early in life with deleterious effects late in life may well apply to the FVL mutation.

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