Evolution of Factor V Leiden

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Summary

Factor V Leiden is a procoagulant mutation associated with venous and arterial thrombosis and pregnancy complications. Its high prevalence of 5% in Caucasians suggests that there are evolutionary benefits as well. Carriers are indeed reported to have various advantageous phenotypes related to haemostasis, inflammation and fertility: less acute blood loss; less menstrual blood loss; decreased risk of intracranial haemorrhage; milder phenotypes of haemophilia; higher survival in and lower susceptibility to severe sepsis; higher survival in acute respiratory distress syndrome; less severe diabetic nephropathy and higher fecundity in both men and women. Not all these associations come from high quality adequately powered studies and many have not been confirmed by further research. The evolutionary influence of the alleged associations varies and is difficult to establish, partly due to a shift over time in risk factors of the diseases concerned. For most of the phenotypes possible mechanistic explanations can be provided. The procoagulant phenotype and perhaps also certain pregnancy complications follow from activated protein C (APC) resistance. Elevated APC levels possibly mediate anti-inflammatory effects. Higher sperm counts and more successful embryo implantation seem to play a role in the increased fecundity.

Keywords

Factor V Leiden, phenotype, reproduction, survival

Introduction

The Factor V Leiden (FVL) mutation is the most common inherited prothrombotic mutation among Caucasians. FV is a coagulation protein that in its active form serves as a cofactor in the conversion of prothrombin into thrombin, resulting in fibrin formation. The FVL mutation is a single nucleotide polymorphism that causes a gain of function in factor Va by rendering it more resistant to degradation by activated protein C (APC). Hence the mutation leads to a prothrombotic state. Indeed FVL is best known as an important risk factor for venous thromboembolism (VTE), e.g. deep venous thrombosis (DVT) and pulmonary embolism (PE) (1). On top of this detrimental attribute, carriers of the mutation are more prone to certain pregnancy complications such as miscarriage (2, 3) and possibly also to coronary artery disease (4, 5).

Around 5% of Caucasians carry the FVL allele. Carriers acquire the mutation probably exclusively by inheritance as opposed to de novo mutations (6). The allele frequency of FVL has evidently risen since its origination, and the allele might currently be in Hardy-Weinberg equilibrium. The Hardy-Weinberg principle states that the allele frequency within a large population will remain constant from generation to generation as long as certain conditions are met (7). These conditions are random mating of individuals, no mixing with other populations, no de novo mutations and no selection of alleles through beneficial or detrimental phenotypes of the allele. While besides the absence of de novo mutations none of these conditions is fully met in the case of FVL, it would seem that the Hardy-Weinberg equilibrium of FVL is clearly disturbed by selection. The allele confers an evident negative selection pressure by causing thrombotic disease and pregnancy complications, which are detrimental to survival and reproduction. A counteracting selection pressure can be assumed to preserve the balance. Carriers were concordantly found to have similar overall mortality (8, 9).

Advantages of FVL indeed exist. A myriad of research has been dedicated to both survival and reproduction benefits associated with the mutation. Survival benefits mainly relate to haemorrhage and inflammation although data are conflicting, especially on inflammation. Nevertheless FVL might be associated with: less acute blood loss, less menstrual blood loss, decreased risk of intracranial haemorrhage, milder phenotypes of haemophilia, higher survival in and lower susceptibility to severe sepsis, higher survival in acute respiratory distress syndrome (ARDS), decreased diabetic nephropathy, improved embryo implantation, a higher sperm count and higher fecundity in both men and women (see Table 1) (10-25).

In order for a genotype to rise or decline in prevalence it ultimately has to affect the number of fertile offspring, either by affecting the mortality at or before the fertile age, or the chances of successful reproduction. Not all of the FVL associated phenotypes...
Table 1: Evolutionary influences of FVL.

<table>
<thead>
<tr>
<th></th>
<th>Estimated effect size of FVL heterozygosity</th>
<th>References</th>
<th>Estimated incidence</th>
<th>Probable trend in incidence since pre-modern time</th>
<th>Relevant to chances of offspring* **</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemostasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>OR: 4.9</td>
<td>(1)</td>
<td>Common</td>
<td>Increased</td>
<td>Yes</td>
</tr>
<tr>
<td>Cerebral vein thrombosis</td>
<td>OR: 4.3</td>
<td>(36)</td>
<td>Rare</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>OR: 1.9</td>
<td>(37)</td>
<td>Uncommon</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Retinal vein thrombosis</td>
<td>OR: 1.7</td>
<td>(38)</td>
<td>Common</td>
<td>Increased</td>
<td>No</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>OR: 1.3</td>
<td>(4)</td>
<td>Common</td>
<td>Increased</td>
<td>Yes</td>
</tr>
<tr>
<td>Ischaemic colitis</td>
<td>Per allele OR: 9.6***</td>
<td>(44)</td>
<td>Uncommon</td>
<td>Increased</td>
<td>No</td>
</tr>
<tr>
<td>Ischaemic stroke in children</td>
<td>OR: 6.0</td>
<td>(50)</td>
<td>Rare</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Porencephaly</td>
<td>25% FVL prevalence in porencephaly</td>
<td>(51)</td>
<td>Rare</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Legg-Calvé-Perthes disease</td>
<td>OR: 3.3</td>
<td>(52)</td>
<td>Rare</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Advantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive intrapartum blood loss</td>
<td>ARR: 0–12%</td>
<td>(13; 14; 53)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Lower menstrual blood loss</td>
<td>1.5–7 g/l higher haemoglobin</td>
<td>(16; 17)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Reduced blood loss</td>
<td>239 ml lower after cardiac surgery</td>
<td>(15)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Spontaneous intracranial haemorrhage</td>
<td>OR: 0.19</td>
<td>(18)</td>
<td>Uncommon</td>
<td>Increased</td>
<td>No</td>
</tr>
<tr>
<td>Haemorrhagic disease of the newborn</td>
<td>-</td>
<td>(59)</td>
<td>Uncommon</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Milder haemophilia phenotype</td>
<td>-</td>
<td>(19)</td>
<td>Rare</td>
<td>Stable</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procoagulant state in sepsis</td>
<td>-</td>
<td>(60–66)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Mortality severe sepsis</td>
<td>RR: 5.0</td>
<td>(75)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Purpura fulminans</td>
<td>RR: 3.1</td>
<td>(77)</td>
<td>Rare</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Advantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival severe sepsis</td>
<td>OR: 2.8</td>
<td>(82)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Susceptibility severe sepsis</td>
<td>RR: 0.86 ***</td>
<td>(72)</td>
<td>Common</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>ARDS mortality</td>
<td>ARR: 42%</td>
<td>(21)</td>
<td>Uncommon</td>
<td>Decreased</td>
<td>Yes</td>
</tr>
<tr>
<td>Protection from diabetic nephropathy</td>
<td>28–83 mg/l lower albuminuria</td>
<td>(22)</td>
<td>Common</td>
<td>Increased</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Fertility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental abruption</td>
<td>4.7</td>
<td>(3)</td>
<td>Common</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>2.2</td>
<td>(3)</td>
<td>Common</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Intra-uterine growth restriction</td>
<td>2.7******</td>
<td>(3)</td>
<td>Common</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Early pregnancy loss (&lt;24 weeks)</td>
<td>1.7</td>
<td>(3)</td>
<td>Common</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Late pregnancy loss (≥24 weeks)</td>
<td>2.1</td>
<td>(3)</td>
<td>Common</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Advantages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecundity females</td>
<td>Time to pregnancy 12 weeks shorter</td>
<td>(25)</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>In-vitro fertilisation success</td>
<td>ARR: −41%</td>
<td>(10)</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Fecundity males</td>
<td>First born within one year RR: 3.5</td>
<td>(24)</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>Sperm count</td>
<td>73 · 10^6 higher******</td>
<td>(11)</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Common: >5 per 10,000 cases per year in general population. Uncommon: 0.1 to 5 per 10,000. Rare: <0.1 per 10,000. ** When present, does the disease or phenotype influence the chances of having fertile offspring, by affecting either the mortality at or before the fertile age, or the chances of successful mating? *** Calculated from published data. **** Without vitamin K prophylaxis. ***** Not statistically significant.
will have had equally significant evolutionary implications. The present review discusses the literature on potential advantages and disadvantages of FVL for survival and reproduction, from an evolutionary point of view. Although the origin of the nucleotide point mutation leading to FVL was not subject to evolution sensu stricto, the penetration of this genetic variation in the genome of the population can be considered as a consequence of evolution. We will first briefly describe the mutation in terms of its origin, its genetic and biochemical features and its prevalence, after which we will discuss the effects of FVL on haemostasis, inflammation and reproduction.

**Factor V Leiden mutation**

FVa is a procoagulant protein. It serves as a cofactor for FXa in catalysing the conversion of prothrombin to thrombin. Thrombin in turn converts fibrinogen into fibrin which is crucial for haemostasis.

The FVL mutation is a missense mutation located in the coding sequence of the FV gene, c.1691G>A. It causes arginine 506, which is one of three cleavage sites for APC, to be replaced with glutamine. The change in amino acid at the cleavage site results in resistance of FVa to inactivation through cleavage by APC (26). The gain-of-function FVL mutation hence facilitates haemostasis and thrombosis.

FV also has an anticoagulant function as a cofactor in the APC-mediated inactivation of FVIIIa (27). FV performs this role only when cleaved at arginine 506. Since FVL is resistant to this cleaving by APC, the mutation has a procoagulant effect in this pathway as well.

Based on haplotype analyses, the mutation is estimated to have occurred 21,000 to 34,000 years ago in one Caucasian individual (28, 29). This age of the mutation is consistent with its racial distribution. The Caucasian subpopulation separated from the other non-African subpopulations around 40,000 years ago (30). The mutation is virtually absent in indigenous populations of continents other than Europe. The carrier rate in Caucasians is reported from 0 to 15% with an average of around 5% (6, 31-33). Fitting explanations for this wide variation are lacking.

**FVL and haemostasis**

**Detrimental traits**

FVL was discovered (34) as a cause for familial cases of APC resistance and venous thrombotic disease (35). Hence the most obvious harm of FVL is the elevated risk of VTE, i.e. DVT and PE, but thrombosis of the cerebral, portal and retinal vein is also more common in carriers (36-38). A large meta-analysis of population-based case-control studies showed that the odds ratio (OR) for VTE is 4.9 (95% confidence interval [CI] 4.1-5.9) when heterozygous for FVL and 9.9 (95%CI 4.8-20.1) when homozygous (1). Interestingly, in this study FVL was more strongly associated with DVT than with PE. Earlier studies came to the same conclusion, finding that the FVL prevalence in DVT patients was almost double that in PE patients (39, 40). In one consecutive series of autopsies in which PE was mentioned in the autopsy report, mostly along with other severe illnesses, the prevalence of FVL was equal to that in the general population (41). The occurrence of fatal PE thus seems at most moderately raised by FVL.

VTE has many other risk factors (42). The presence of some of these factors, especially the acquired ones, has not been constant over time. Old age, immobilisation and malignancy have always been around but have presumably become more prevalent. Other risk factors were introduced in modern times such as surgery, long distance travel, obesity, oral contraceptive use, hormone replacement therapy and central venous catheters. The rise in all these risk factors leads us to assume that the incidence of VTE must have also risen since pre-modern times, even more so because the risk factors can have a synergistic effect when they occur simultaneously (1, 43).

A small but significant association with arterial thrombosis also exists according to two meta-analyses. The OR for acute myocardial infarction with FVL was 1.29 (1.03 - 1.61) (4). The per-allele relative risk (RR) for coronary heart disease was 1.17 (1.08 - 1.28) (5). Besides coronary artery disease, an association of FVL with ischaemic colitis is also reported (44). The rarity of this disease at and before fertile age minimises the evolutionary implications (45). The relation of FVL with ischaemic stroke is controversial, especially in young adults (46). A meta-analysis did not show a significant correlation (47).

One possible explanation for the association with arterial thrombosis being much smaller comes from an experimental study using a mouse model to investigate atherogenesis. Although plaques were larger in the FVL mice, they were morphologically more stable and did not increase stenosis because of positive vascular remodelling (48).

The same arguments for the limited influence of PE on FVL evolution apply even more strongly to arterial thrombosis. While the association does exist, it is very modest. Furthermore the many other risk factors for arterial thrombosis were likely less prevalent in prehistoric times.

In children associations have been reported between FVL carriership and VTE, ischaemic stroke and porencephaly, which often also has a thrombotic pathogenesis (49-51). These are all potentially lethal illnesses. Child mortality has major evolutionary impact. However the incidence of these respective conditions lies around 0.5-5 in 100,000. There is also a controversial relation of FVL with Legg-Calvé-Perthes’ disease, in which osteonecrosis of the proximal femoral epiphysis is believed to be caused by thrombosis (52).

**Beneficial traits**

An obvious hypothesis when searching for potential mechanisms of positive selection would be that the procoagulant FVL mutation leads to a reduction in blood loss. As detailed below, carriers are found to have a lower tendency to bleed, or tend to have a lower volume of blood loss in several situations.
In two Swedish cohorts of women who delivered, one pro- and one retrospective, there was an absolute risk reduction of 4% and 12% respectively, for intrapartum blood loss exceeding 600 ml (13, 14). This finding was later contradicted by another adequately powered prospective study in which the difference between carriers and non-carriers in blood loss was not significant (53). In the Swedish studies blood loss was measured, whereas in this last study, the outcome was defined as blood loss visually estimated to exceed 500 ml, requiring intervention.

In one of the Swedish cohorts, FVL carriers had higher values of haemoglobin and ferritin early in pregnancy as well as less self-reported menstrual blood loss (16). Another group reported a difference between FVL carriers and non-carriers in haemoglobin values in pre-menopausal women, whereas the difference was absent in men and post-menopausal women (17). These findings suggest lower menstrual blood loss in FVL carriers. Both reduced peripartum blood loss and higher haemoglobin values antepartum would be of tremendous evolutionary impact, since haemorrhagic pregnancy complications contributed considerably to maternal mortality in times preceding modern medicine (54). Haemorrhage is still the most important cause of maternal death in the developing world (55).

Reduced blood loss in FVL carriers was demonstrated in a prospective cohort of patients undergoing cardiac surgery (15). After 24 hours the chest tube output was 239 ml (p=0.007) lower compared to non-carriers, corrected for other significant confounders.

Adult FVL carriers were also found to have an OR of 0.19 (95%CI 0.03-.095) for spontaneous intracranial haemorrhage in a prospective case-control study (18). This finding reflects the beneficial effect of FVL in the balance between haemorrhage and thrombosis. However, it has little effect on survival up to reproductive age since the mean age of the subjects was 66 years.

In very-low-birth-weight neonates a protective effect of FVL against higher grades of intraventricular haemorrhage, which are defined by extended bleeding, was reported by one retrospective cohort study (56). However, the relation seems unlikely, since the published data dubiously support the conclusion and two prospective studies reported no or even an opposite association (57;58). Since intraventricular haemorrhage particularly affects very-low-birth-weight neonates which have a very limited chance of survival without modern medicine, the evolutionary implications of this alleged association are marginal. The question whether FVL protects neonates from bleeding remains important, however, since haemorrhagic disease of the newborn, which is caused by vitamin K deficiency, can be presumed to have been more prevalent in pre-modern times (59). Diet was possibly less rich and/or constant in vitamin K. Furthermore, breastfeeding, which is a major risk factor for vitamin K deficiency, was more common than it is nowadays.

Analogous to the sickle cell gene of which heterozygosity confers a survival advantage in malaria, FVL was hypothesised to give milder phenotypes in hereditary bleeding disorders such as haemophilia disease. Several studies have indeed found a reduced bleeding tendency in haemophiliacs carrying FVL, while others rejected the hypothesis (19). The prevalence of haemophilia lies around 1-2 in 10,000 males. The evolutionary implications of this contested benefit would have been marginal.

**FVL and inflammation**

**Detrimental traits**

The relation of FVL to inflammation is less discernible than to haemostasis. Yet, much research has been performed on the effect of FVL and the protein C (PC) pathway on inflammatory processes. In general, defects in the PC mechanism enhance the vulnerability to systemic inflammatory reactions. This is probably mediated at least for a large part through more severe inflammation-induced coagulation. In patient studies lowered levels of PC and protein S (PS) are associated with increased mortality (60-62). Mice with a one allele targeted disruption of the PC gene, causing heterozygous PC deficiency, displayed a more severe disseminated intravascular coagulation and associated inflammatory response (63). Low levels of free PS (the co-factor of APC) further compromise an adequate function of the PC system. In plasma, 60% of the co-factor PS is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP as a consequence of the acute phase reaction in inflammatory diseases may result in a relative PS deficiency contributive to a further procoagulant state during sepsis. Although it has been shown that the β-chain of C4bBP (which mainly governs the binding to PS) is not very much affected during the acute phase response (64), support for this hypothesis comes from studies showing that the infusion of C4bBP in combination with a sublethal dose of *Escherichia coli* into baboons resulted in a lethal response with severe organ damage due to disseminated intravascular coagulation (DIC) (65). Animal experiments of severe inflammation-induced coagulation activation convincingly show that compromising the PC system results in increased morbidity and mortality, whereas restoring an adequate function of APC improves survival and organ failure (66). Part of these processes may be mediated by APC effects on coagulation. Hence FVL, which leads to impaired effects of APC on coagulation, may deteriorate the inflammatory state.

**Beneficial traits**

One important study to this field was the PROWESS trial (67), a phase three trial assessing the efficacy of recombinant human activated protein C (rhAPC) for severe sepsis. The study population in this trial was also used to test the hypothesis that FVL confers survival advantage in severe sepsis. The corrected odds of survival past 28 days was indeed almost three times higher for FVL carriers (p=0.006) (20). The hypothesis was further tested in a mouse endotoxaemia model in the same paper. The authors reported a three times lower mortality of FVL heterozygous mice compared to wild-type mice (p=0.008).

Later animal experimental studies of comparable design have not been able to reproduce this survival benefit. FVL hetero- or
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homzygosity was not associated with survival in mouse models mimicking bacterial peritonitis, group A streptococcal subcutaneous infection, pneumococcal pneumonia and influenza A pneumonia (68-71). The allele also did not significantly influence bacterial outgrowth, inflammatory markers and coagulation, although influenza A viral load was higher with FVL.

More human research on FVL and inflammation has been performed. Data from the aforementioned PROWESS trial were pooled with data from the ENHANCE study, a large prospective cohort study in patients with sepsis treated with rhAPC, to further investigate susceptibility to and mortality from sepsis (72). The prevalence of FVL amongst septic patients was slightly lower than predicted with a p-value of 0.05, pointing towards a lower susceptibility to severe sepsis. However, the higher survival of FVL carriers was not statistically significant when the PROWESS data were pooled with those of the ENHANCE study (RR 0.74, 95%CI 0.53-1.03). Differences in mortality between carriers and non-carriers were also not found in two other studies in septic children and adults, respectively (73, 74). Both these studies were underpowered, and reporting in the study with septic children was poor. Two studies by a Danish group even found some detrimental effects of FVL on infectious disease susceptibility and mortality. The first, a prospective cohort study investigating multiple associations with FVL, found a five times increased mortality in carriers with sepsis (75). When corrected for multiple comparisons, the association did not remain significant. The incidence of sepsis was not increased. The second study did not find an overall elevated susceptibility to or mortality from certain critical illnesses in FVL carriers (76). However, FVL frequency was slightly higher in cases of intensive care unit admission compared to controls, and in cases that died in that subgroup. Finally, a retrospective cohort study investigated 259 children with meningococcal sepsis and meningitis (77). Mortality did not differ according to carrier status statistically, but the study was not powered to this end. FVL carriers did have a three times higher risk of purpura fulminans, a complication of meningococcal infection with thrombotic pathogenesis.

Effects of FVL mutation on non-infectious inflammatory pathology have also been studied. In a cohort of retrospectively selected acute respiratory distress syndrome (ARDS) patients, mortality was lower in carriers compared to non-carriers (p=0.049) (21). Although ARDS is in itself not an infectious disease, the syndrome is often caused by sepsis or pneumonia as was the case in this study in all FVL carriers. Since the results were not corrected for underlying diseases the survival advantage associated with FVL might apply to sepsis and pneumonia instead of ARDS. FVL has also been reported to protect against diabetic nephropathy (22). This conclusion was based on lower levels of albuminuria in diabetic mice and humans with FVL, and histologically less severe nephropathy in the mice.

If FVL indeed renders carriers better equipped against inflammatory disease, albeit only in certain circumstances, then a possible explanation lies in protective effects of APC. The resistance of the FVL enzyme to degradation by APC might be assumed to elevate levels of APC, by feedback through thrombin and thrombomodulin. This is supported by elevated levels of APC-PC inhibitor complex in FVL carriers with a history of VTE (78). An earlier underpowered study found non significantly elevated APC levels in healthy carriers (79). APC has effects on several facets of inflammation besides the inflammation-induced coagulation described above. Almost all these effects are dependent on the activation of PAR1, by APC binding to endothelial PC receptor. This ligation induces anti-inflammatory processes, anti-apoptotic processes, gene-expression alterations and improved endothelial barrier function, through mechanism which are only partly unravelled (80, 81).

The presence of a protective inflammation-related effect of APC was demonstrated in an elegant experiment comparing survival of FVL mice and mice with impaired thrombomodulin function, submitted to an endotoxin challenge (82). FVL and impaired thrombomodulin have the same overall effect on coagulation through a disruption of the APC pathway. However, the effect on APC is opposite. Where FVL increased APC formation, the impaired thrombomodulin decreased it. This experimental setup allows studying the non-coagulation effects of APC. FVL mice had a relatively low mortality compared to wild-type. Mice with reduced thrombomodulin function had 100% mortality after the same endotoxin challenge. This suggests a protective non-coagulation related effect of APC. In an earlier study septic baboons that were injected with APC had a higher survival than those injected with saline (83). Furthermore, in humans decreased levels of APC have been reported to correlate with sepsis and with death from sepsis (84-86).

All these observations formed the basis for the hypothesis supporting the administration of rhAPC to septic patient in the PROWESS trial. In the PROWESS trial treatment indeed resulted in an improved survival from sepsis. However, a Cochrane systematic review from 2012 that included five randomised controlled trials (RCTs), did not confirm this (87). The recently published PROWESS-SHOCK trial found similar survival in the rhAPC treated and untreated group, finally leading to the withdrawal of rhAPC from the market (88).

To summarise, research in both animals and humans has suggested several effects of FVL, and relatedly APC, on inflammation. A detrimental trait seems to be the procoagulant state predisposing to inflammation-induced coagulation. Several beneficial traits have also been proposed. Firstly, FVL carriers were reported by one study to have a survival benefit in severe sepsis. This was not validated by further studies, in sepsis, nor in other infectious diseases. Secondly, susceptibility of carriers to sepsis might possibly be lower. Thirdly, FVL might have a positive influence on some non-infectious inflammatory processes. And lastly, rhAPC treatment initially appeared to have a protective effect in sepsis, providing a possible mechanistic explanation for any advantages of FVL in inflammation, assuming FVL elevates APC levels. The treatment benefit was, however, finally dismissed by more recent trials. One explanation for these heterogeneous findings could be that the net effect of FVL on inflammation is dependent on any number of unidentified factors, for example type of infection, occupancy of certain receptors or other biochemical factors. FVL’s effect on sepsis outcome might be nullified by other more profound
alterations in coagulation during sepsis (89). Methodological aspects might have had influence, though can hardly be imagined to account for all reported differences. Publication bias could also play a role given the temporal order of the positive and negative study results, making it possible that there are in fact very little or no beneficial effects of FVL on inflammation.

### FVL and reproduction

#### Detrimental traits

Reproduction, not survival, is ultimately the driving force behind evolution. FVL seems to exert ambiguous effects on reproduction through diverse and largely unknown mechanisms.

Several systematic reviews have been devoted to the question whether FVL is associated with certain pregnancy complications. Most have found a positive answer to this question. The highest quality and second largest meta-analysis on the subject found the following ORs: placental abruption 4.7 (95% CI 1.1 – 19.6), pre-eclampsia 2.2 (95% CI 1.5 – 3.3), intra uterine growth restriction 2.7 (95% CI 0.6 – 12.1), early pregnancy loss (<24 weeks) 1.7 (95% CI 1.1 – 2.6) and late pregnancy loss (≥24 weeks) 2.1 (95% CI 1.1 – 3.9) (3). These are not corrected for confounders. The studies included in the meta-analysis were case-control studies which tend to overestimate associations because of selection bias.

#### Beneficial traits

Interesting results were found in a study examining the fecundity among male and female FVL carriers who had their fertile years before contraceptive methods were in use (24). Fecundity was estimated by establishing the time between a person’s marriage and the birth of the first child. The men carrying FVL had a RR of 3.5 for having a first born within one year of marriage. The female carriers had neither an increased nor a decreased fecundity measured in this way. In light of the elevated risk for fetal loss associated with FVL, this suggests that female carriers became pregnant more quickly. The carriers had more fetal losses yet they had equally as many live births within one year.

A recent study by our own group supported the higher fecundity in female FVL carriers (25). The cohort consisted of women with unexplained recurrent miscarriage included in the ALIFE trial, a RCT that evaluated anticoagulant treatment to improve live birth. The women who were not pregnant at time of randomisation were included in an analysis with time to pregnancy as primary outcome. The median time to pregnancy for FVL carriers was 11 weeks (interquartile range [IQR] 6 - 21) while it was 23 weeks (IQR 8 - 56) for non-carriers (p=0.032). The association remained significant after correction for potential confounders.

Another suggestion in the direction of high fecundity in FVL carriers was a reported prevalence of 9.2% in healthy pregnant Dutch women, substantially higher than in the non-pregnant population (90).

Two publications on success rates of in-vitro-fertilisation (IVF) provided insight into possible mechanisms behind the fecundity effects. IVF can be regarded as a model for embryo implantation. The first group found that the first attempt IVF embryo transfer, preceded by intra-cytoplasmic sperm injection, was successful in 90% in mother-child pairs carrying FVL, versus 49% in the control group (p=0.018) (10). The second group investigated whether routine testing for FVL was indicated in IVF populations (91). A preliminary analysis showed a lower than expected prevalence of FVL (1.6%) in this subfertility population and a doubled success rate in carriers (p=0.08). Both these findings suggested an unexpected positive effect of FVL on fertility. This was reason to stop the study, prior to reaching statistically significant results. However a trend towards higher implantation success rates in carriers was clear.

Possibly contributing to the aforementioned high fecundity in FVL males is a statistically non-significant elevation in sperm count. In a cohort of men of subfertile couples, total sperm count was 236 · 10⁶ (95%CI 158 - 292 · 10⁶) in FVL carriers versus 163 · 10⁶ (95%CI 147 - 178 · 10⁶) in controls (11).

### Conclusion

The high prevalence of FVL in Caucasian populations implies that its detrimental traits are balanced by the associated beneficial phenotypes. Of the diseases FVL is reported to affect, not all are likely to have been of notable evolutionary importance. This was either because the effect of FVL was minimal; because having the disease did not affect the chances of successful mating; or because the disease was not prevalent amongst fertile individuals in pre-modern times (see Table 1). Respective examples are portal vein thrombosis, retinal vein thrombosis or diabetic nephropathy. The reported FVL-associated phenotypes that seem most likely to have had considerable evolutionary impact would be: protection from acute blood loss, menstrual blood loss and haemorrhagic disease of the newborn; higher fecundity and proneness to pregnancy complications, coronary heart disease and venous thromboembolism. The selective pressure conferred by PE has possibly not been as large it might appear because the risk of fatal PE seems at most moderately raised by FVL and other risk factors were much less prevalent in earlier times.

Not fitting into the raised categories in this review, the prevalence of FVL in children of the Dutch Famine Birth Cohort suggested the possibility of a survival advantage during famine (92).

Definitive explanations are mostly lacking, but for many phenotypes possible mechanisms are suggested. Higher fecundity might partly result from higher sperm counts and more successful

### Abbreviations

FVL, Factor V Leiden; PC, protein C; APC, activated protein C; rhAPC, recombinant human activated protein C; PS, protein S; VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; ARDS, acute respiratory distress syndrome; IVF, in-vitro-fertilisation; OR, odds ratio; CI, confidence interval; RR, relative risk; ARR, absolute risk reduction; IQR, interquartile range.
embryo implantation. The procoagulant phenotype and perhaps also certain pregnancy complications follow from APC resistance and elevated thrombin production. If FVL indeed has protective effects on inflammation, this could be mediated by elevated APC levels.

High quality adequately powered prospective cohort studies on the associations between FVL and the discussed diseases are scarce. Furthermore, reproducibility of associations between FVL and certain advantageous phenotypes has been poor, with exception of those relating to reproduction. Effects of FVL on inflammation are especially inconsistent. In many cases a first study reported a beneficial effect of FVL, which was then contested by further studies. It is not unlikely that publication bias is a problem in this field of research driven by the attractive hypothesis of existence of evolutionary benefits.

Conflicts of interest
None declared.

References


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