Thrombophilia ad dies vitae

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Chapter 12

Increased sperm count maintains high population frequency of Factor V Leiden

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Abstract

Background

Factor V Leiden (FVL) increases the risk of venous thrombosis and pregnancy loss in carriers. Nevertheless, this relatively old mutation is prevalent in Caucasian populations, which could be explained by positive selection pressure. Men with FVL have previously been found to have higher fecundity (the time between marriage and first pregnancy). Whether this is caused by increased sperm counts in men with FVL is unknown.

Objectives

To assess whether men with Factor V Leiden have increased sperm counts.

Methods

We performed a prospective cohort study among 1139 consecutively included male partners of subfertile couples presenting at our university hospital for fertility workup between January 2000 and July 2007. All potential candidates who gave informed consent were included, irrespective of their fertility workup. In this retrospective analysis, we excluded participants with known causes of spermatogenic function or azoospermia. Subsequently, we genotyped all participants and compared sperm counts between FVL carriers and non-carriers.

Results

We identified 37 FVL carriers and 921 non-carriers. FVL carriers had higher total sperm counts and total motile sperm counts than non-carriers: 236 $x10^6$ (95%CI 158-292 $x10^6$) versus 163 $x10^6$ (95%CI 147-178 $x10^6$) and 81 $x10^6$ (95%CI 54-105 $x10^6$) versus 52 $x10^6$ (95%CI 48-57 $x10^6$), respectively.

Conclusion

Our results provide a possible explanation for the high prevalence of FVL among Caucasians. To our knowledge, this is the first study that indicates that an increased prevalence of a genotype is controlled by *increased* sperm counts.

Introduction

Factor V Leiden (rs6025, FVL) is the most common inherited thrombophilic defect in Western countries.[1] This gain-of-function mutation leads to resistance of activated clotting factor V through inactivation by activated protein C.[2,3] Carriers are at increased risk of venous thrombosis and spontaneous or recurrent miscarriage.[3,4]

Despite these obvious disadvantages, the mutation -which occurred about 21,000 - 34,000 years ago[5]- has a high prevalence of approximately 4-7% in Caucasians.[6] It has long been thought that the high population frequency of FVL reflects some sort of evolutionary benefit for carriers.[7-11] Interestingly, male, but not female FVL carriers display a higher fecundity rate (the time between marriage and first pregnancy) than non-carriers.[12] We hypothesized that men with FVL have high sperm counts thereby increasing their chance to establish a pregnancy and spreading their genotype.

Methods

Pilot study

We performed a pilot study between August and November 2008 among nineteen male FVL carriers, who had been tested for the mutation in a previous study that investigated the associated risk of venous thrombosis[13] or who had been tested for clinical purposes because of a history of venous thrombosis. These participants donated two semen samples with a two-week time interval, each after three or more days of sexual abstinence, at the fertility laboratory of the Academic Medical Center, Amsterdam, the Netherlands. The technicians who performed the semen analyses were unaware of the participants' FVL status. Donated semen was analyzed for volume, sperm concentration, sperm motility and sperm morphology according to the WHO guidelines.[14] Total sperm count and total motile sperm count were calculated. The outcomes were compared to previously published cohorts of the general population.[15-18]

Cohort study

Participant identification

Subsequently, we performed a formal cohort analysis. We genotyped a cohort of consecutive male partners of subfertile couples presenting at the Center for Reproductive Medicine of the Academic Medical Center for a fertility workup, from January 2000 until July 2007. All men were consecutively included prior to semen analyses. None of the participants of the pilot study had also been included in this cohort study. The study was

approved by the Institutional Review Board of the Academic Medical Center. Written informed consent to store and use their DNA for research purposes was obtained from all men.

Exclusion criteria

We excluded men with known causes of spermatogenic failure: i.e. hyperprolactinaemia, hypogonadotrophic hypogonadism, previous chemo- or radiotherapy, bilateral cryptorchidism, congenital absence or surgery of the vas deferens, history of orchitis, and bilateral orchidectomy. Participants were also excluded from this analysis if the fertility workup identified retrograde ejaculation, azoospermia, an *AFZa*, *P5/proximal-P1*, *P5/distal-P1, AZFc* or *gr/gr* deletion, or numerical or structural chromosome abnormalities.

PCR analysis

The FVL mutation (G1691A) was identified by PCR on a Biorad CFX96 or Roche Lightcycler 480 Real Time System. The laboratory technicians who performed the PCR analyses were unaware of the results of the semen analyses.

Statistics

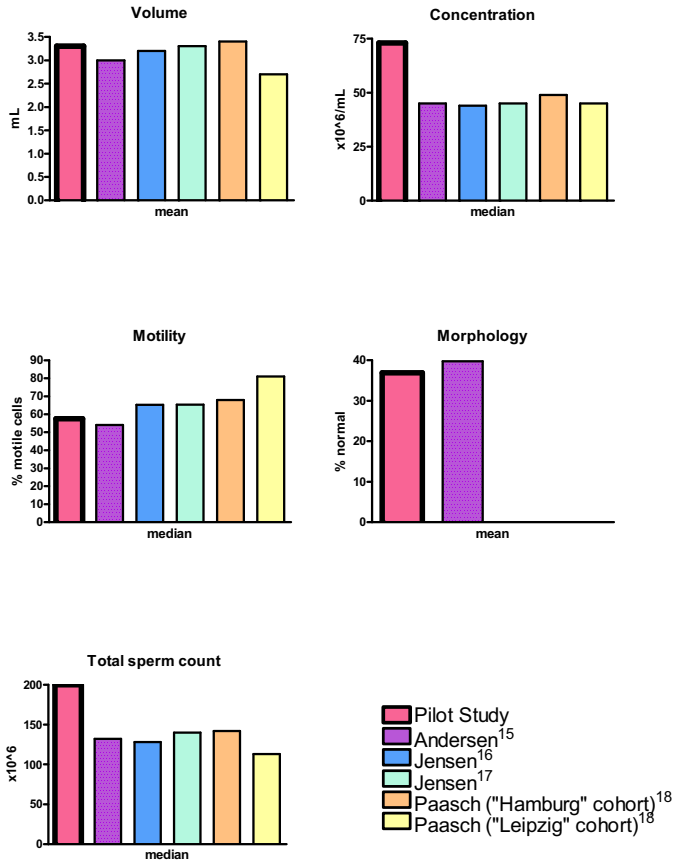
The average of the participant's two or more semen analyses was included in the statistical analysis. In case of normally distributed data, we calculated means and 95% confidence intervals using SPSS version 16.0. For non-normally distributed data, we calculated median values and 95% confidence intervals by performing bootstrap procedures in "The R Project for Statistical Computing", available at: http://www.r-project.org/.

Results

The pilot study suggested that FVL carriers had increased sperm concentration (median 75 x10^6/mL, 95%CI 58 - 95 x10^6/mL) and increased total sperm count (median 192 x10^6, 95%CI 132 - 247 x10^6) as compared to previously published cohorts of the general population (see Figure 1).[15-18] We also assessed total motile sperm count: median 77 x10^6 (95%CI 55 - 105 x10^6), however, this parameter could not be compared to the general population, as it had not been reported.[15-18]

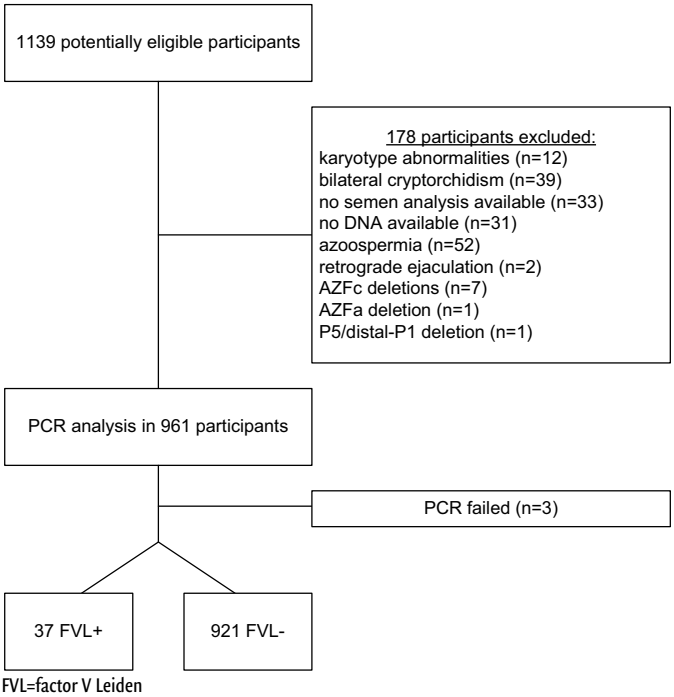
Of 1139 screened participants of the cohort study, 181 met at least one exclusion criterion (for reasons of exclusion: see Figure 2). At least two semen samples were available for each participant. We identified 37 men heterozygous for FVL and 921 men without the mutation.

Figure 1. Semen analyses of 19 factor V Leiden carriers (pilot study) as compared to the general population[15-18]



As depicted in the Table, median total sperm count was increased in FVL carriers as compared to non-carriers: 236 x10^6 (95%CI 158-292 x10^6) and 163 x10^6 (95%CI 147-178 x10^6), respectively. In addition, we found increased total motile sperm counts in FVL carriers as compared to our controls: 81 x10^6 (95%CI 54-105 x10^6) versus 52 x10^6 (95%CI 48-57 x10^6).

Figure 2. Flow chart of the cohort study



FVL=factor V Leiden

Table. Semen analyses of 37 carriers of the factor V Leiden mutation as compared to 921 non-carriers

	FVL +	FVL -
participants (n)	37	921
mean age (y)	36 (34 - 38)	37 (36 - 37)
mean days of sexual abstinence (n)	4.0 (3.5 - 4.5)	4.2 (4.1 - 4.4)
	semen quality	
mean volume (mL)	3.7 (3.3 - 4.2)	3.4 (3.3 - 3.5)
median concentration (10^6/mL)	58 (47 - 80)	56 (52 - 60)
median motility (% progressive)	36 (28 - 41)	34 (32 - 35)
mean morphology (% normal)	38 (34 - 43)	35 (34 - 36)
median total count (10^6)	236 (158 - 292)	163 (147 - 178)
median total motile count (10^6)	81 (54 - 105)	52 (48 - 57)

FVL= factor V Leiden. Means and medians are presented with 95% confidence intervals

Discussion

Our data show that men with FVL have increased total sperm counts and total motile sperm counts. The observed high frequency of FVL among the population could thus be the evolutionary result of a balance between allele loss through thrombosis and pregnancy loss, and allele gain through increased male fertility. Higher sperm counts are likely to increase the chance of a successfully established pregnancy, as lower sperm counts are associated with decreased fertility. However, no studies have yet focused on determinants of increased male fertility.

Our results provide an explanation for the increased fecundity found in men with FVL as described in a previous study.[12] In this large case-control study among 1176 subjects, the relative risk of conception within 3 months after marriage was 3.5 (95%CI 2.1-5.7) for men with FVL as compared to men without FVL.

Also, a biological explanation may be possible for the association between FVL and higher sperm counts, even though a direct function of the factor V protein in spermatogenesis has not been investigated. However, two studies have found an excess of breakpoints in chromosome 1, including the FVL locus on 1q23, among infertile males.[19,20] Moreover, two infertile brothers with a pericentric inversion of chromosome 1 including the FVL locus (p34q23) were shown to suffer from severe oligozoospermia.[21] Thus, it could be that the 1q23 locus harbours an important gene for spermatogenesis.

Our data suggest two directions for future research. First, an investigation into reasons for increased fertility and fecundity, in particular higher sperm counts, is needed. Second, the biological explanation of the relationship between FVL and high sperm counts may offer an opportunity to gain more fundamental insight into male fertility regulation.

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