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The Factor V Leiden mutation is associated with a higher blood haemoglobin concentration in women below 50 of the Malmö Thrombophilia Study (MATS).

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Short title: Factor V Leiden, women and haemoglobin.

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Abstract

Objectives:

The aim of this study was to investigate a relationship between FVL-mutation and levels of haemoglobin (Hb) in patients with venous thromboembolism (VTE).

Patients and methods:

From March 1998 to December 2005, 927 consecutive patients with objectively diagnosed VTE were registered in the Malmö Thrombophilia Study (MATS).

Results:

Female patients with FVL-mutation below 50 years of age had significantly higher median-Hb (133 g/l versus 126g/l; $p < 0.001$) compared to female patients below the age of 50 years without FVL. No significant difference could be found for men or women above 50 years of age or men below 50 years of age.

Conclusion:

Female patients below the age of 50 years with FVL-mutation and VTE are associated with higher median Hb, and this finding is in accordance with earlier hypothesis that FVL-mutation may have constituted an evolutionary selection advantage.

Key words:

Factor V Leiden, Haemoglobin, thrombosis, women

Introduction

Factor V Leiden mutation (FVL) is thought to have occurred in one individual some 21.000 to 34.000 years ago [1]. Hemorrhagic complications have always existed in human history. In 17th century London, pregnancy was associated with approximately 2% increased death risk, and mortality risk during pregnancy was 10%, where severe haemorrhage during labour and delivery was a major cause of mortality [2]. Since the FVL-mutation is still prevalent in around 5% in the Caucasian populations [3-4], one may speculate that it constituted an evolutionary selection advantage by avoiding anaemia or perfuse blood loss during childbirth in ancient times.

Since the discovery of APC resistance and the FVL in 1993 [5, 3, 6], knowledge about hereditary thrombophilia has increased remarkably. We now know that venous thromboembolism (VTE) is a multi-causal disease which occurs often when genetic and acquired risk factors are present at the same time [7].

The FVL-mutation is one of the most common genetic risk factors for VTE, and approximately 5% of the Caucasian populations are carriers of this mutation [3-4]. More recently it has been reported that the prevalence of FVL-mutation in European populations is approximately 3% to 7% with rare occurrence in black and Asian populations [8].

Several clinical analyses of pregnant women with FVL-mutation indicate a reduced risk of intrapartum bleeding, and significantly less intrapartum blood loss compared to women without the mutation [9-10]. Women with FVL-mutation were also characterized by higher postpartum haemoglobin (Hb) values, less risk of postpartum anaemia [9], higher Hb-values and ferretin values in early pregnancy, and lower self-estimated menstrual blood loss [11]. Furthermore, studies in other patients groups with FVL-mutation undergoing cardiac surgery show significantly lower blood losses at 6 and 24 hours compared to non-carriers [12].

Hence, we found it interesting to investigate whether we could find a relationship between FVL-mutation and Hb levels in 927 patients in the Malmö Thrombophilia Study (MATS). We chose to study women in pre and post menopausal age in particular.

Patients and methods

Patients

The Malmö Thrombophilia Study (MATS) is a prospective study undertaken during March 1998 – December 2005. The criteria for including a patient in MATS are: that the patient is over 18 years of age, can communicate in Swedish and that the thrombosis is diagnosed at Malmö University Hospital, with an objective method such as phlaebography, computed tomography, magnetic resonance imaging or ultrasound. Patient history, acquired and genetic risk factors for VTE are available for each patient, along with standard clinical laboratory tests. All patients with objectively diagnosed VTE in MATS were treated in accordance with the standard protocol of Malmö University Hospital. The protocol suggests for the first VTE, 3-6 months of therapy with warfarin, and for recurrence of VTE to consider longer term treatment. All patients are treated with LMWH or heparin during the initiation of warfarin treatment. The rate of consensual participation in MATS is estimated to be about 90%. Remaining 10% of patients were excluded du to language problems and unwillingness to participate in MATS. As of December 2005, the cohort contained 1056 patients, of which 927 have been analysed for carrier ship of the FVL-mutation. To each of these patients, a characteristic Hb was assigned by computing the median of all Hb values available for that patient in the Malmö University Hospital database records. For 95 patients there was just one Hb value and for 340 patients there were more then 10 values. The median number of Hb values per patients was 7. This characteristic Hb will henceforth be referred to as the patients Hb. Since data for menopausal onset were not readily available, we used an age limit of 50 year or lower to characterise the group of pre menopausal women. The study was approved by Lund University Ethical Committee.

Laboratory analyses

Patients in our study were tested for APC resistance by the COATEST®, APC™ RESISTANCE V (Chromogenix) according to the manufacturers' instructions until the year of 2000. All patients with APC resistance ratios < 2.0 were analysed for the FVL-mutation as previously described [3, 13]. After the year 2000 all included patients were tested with a DNA test för FVL. Carrier ship of FVL was defined as either heterozygous or homozygous. Blood haemoglobin was automatically analyzed on a Coulter CH 750 System analyzer (Beckman Coulter Inc; CA USA).

Presence or absence of the prothrombin gene 20210 G to A transition was determined as described previously (Poort SW 1196).

Statistical analyses

All statistical analyses were performed using GNU R [14] on a Macintosh Powerbook G3, running Debian GNU/Linux version 3.1. To avoid the effects of outliers, small samples, and non-normality of distributions we employed medians and Mann-Whitney U tests for statistical characterization and testing. P values <0.05 were considered to indicate statistical significance.

Results

The overall prevalence of FVL-mutation in our cohort of VTE patients was found to be 288 (31%), of which 261 were heterozygous for the FVL-mutation, and 27 homozygous. Patient characteristics, with respect to Hb, of the MATS cohort are shown in Table 1. Female patients with FVL-mutation below age of 50 years had significantly higher median-Hb (133 g/l versus 126 g/l; $p < 0.001$) compared to female patients below the age of 50 years without FVL-mutation. No significant difference was found for women with and without the FVL-mutation and Hb above the age of 50 years. Furthermore in female patients below the age of 50 years with FVL-mutation the number of heterozygous was 30 and seven were homozygous. There were no significant differences regarding median-Hb between these two subgroups. There were no significant differences in Hb value for men with and without FVL-mutation when studying men below and above the age of 50 years, separately.

Patients with FVL-mutation had significantly lower median age compared to patients with without FVL-mutation (67 years versus 70 years; $p = 0.024$). Male carriers of the FVL-mutation had median age 65 years, compared to 70 years for non carriers ($p = 0.004$). For women, the median ages did not differ (70 years versus 70 years; $p = 0.687$) between groups.

The overall prevalence of prothrombin gene mutation in this cohort was 46 of 916 (5%) patients tested for the mutation. A similar analysis of Hb-values in relation to prothrombin gene mutation and age over and below 50 years was performed for men and women without any significant differences (data not shown).

Discussion

Our study has shown that female carriers of the FVL-mutation in premenopausal age have significantly higher Hb values than non carriers. This is in good agreement with previous studies of Hb levels in pregnant women done by Lindqvist et al 1998, 1999, 2001 [9-11]. More over the study by Lindqvist et al 1998 [9] showed that FVL-mutation women had less intrapartum blood loss and lower risk of severe intrapartum bleeding compared to non-carriers. In fact APC-resistant pregnant women were also characterized by significantly higher postpartum Hb value.

However, the women in our cohort were selected for laboratory thrombophilia investigation due to VTE and thus the cohort is not entirely representative of the general population. There may of course be other VTE associated risk factors such as prothrombin mutation, which may influence blood haemoglobin concentrations. We look for the effect of the prothrombin mutation in our cohort on Hb values but could not find any significant differences. This could be due to a power problem caused by the small number of patient with the prothrombin gene mutation in the cohort.

Furthermore the warfarin treatment could potentially increase the menstrual blood loss in pre menopausal women without the FVL-mutation. Eighteen out of 69 patients (26%) in the group of pre menopausal women lacking the FVL-mutation had Hb values below our reference interval of 117 g/l. Excluding these 18 patients, and two out of 37 (5%) patients based on the same criteria from the FVL group there was still a significant difference ($p < 0.032$).

We speculate that the FVL-mutation may serve as a protection against profuse menstrual blood loss since there are no differences between the groups of FVL-mutated women below 50 and women above 50 with and without the mutation.

Several studies suggested beneficial effects of the FVL-mutation. Brian S et al 2003 [12] have shown decreased blood loss during cardiac surgery in patients carrying the FVL-mutation, and recently published studies, on both mice and men, show beneficial effects of the FVL-mutation on the outcome of severe inflammatory diseases and sepsis [15-17].

It may be speculated that the FVL-mutation constitutes an example of balanced gene polymorphisms, exerting both positive and negative evolutionary pressure. The extent of positive pressure may have been greater before the age of modern medicine as many conditions causing profuse blood loss can be treated today.

In conclusion, VTE female patients below age 50 with FVL-mutation are associated with higher median Hb compared to female patients without hereditary thrombophilia and may support earlier hypothesis that FVL-mutation may have had evolutionary selection advantage over time by avoiding bleeding.

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Table 1. Population characteristics of the MATS cohort with respect to FVL-mutation and blood haemoglobin value measured in grams per litter. The numbers in each cell denote median blood Hb value and the numbers within parenthesis denote the number of patients in each category. The last column contains p-values from comparison between the FVL-mutation and non FVL-mutation populations.

	All	FVL	Non FVL	p-value
	Hb (g/l)	Hb(g/l)	Hb (g/l)	
	(patients)	(patients)	(patients)	
All	136 (n=927)	137 (n=288)	135 (n=639)	0.054
Male	144 (n=454)	144 (n=143)	143 (n=311)	0.394
Female	131 (n=473)	132 (n=145)	130 (n=328)	0.182
Female <50	128 (n=106)	133 (n=37)	126 (n=69)	0.001