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Homozygous factor V Leiden and double heterozygosity for factor V Leiden and prothrombin mutation

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Abstract The most common forms of familial thrombophilia are factor V Leiden (FVL) and prothrombin mutation (PTM). Homozygous FVL and PTM have long been feared conditions thought to cause high rates of morbidity and mortality. To analyse clinical features in patients with homozygous FVL and PTM, as well as patients with double heterozygosity for FVL and PTM. All patients with homozygous FVL, PTM or double heterozygosity in the MATS database of 1465 consecutive unselected patients were analysed regarding age at inclusion venous thromboembolism (VTE), age at first thrombosis, recurrence, clinical course and acquired risk factors. We found 36 patients homozygous for FVL. Patients homozygous for FVL were younger than controls at group level (56 ± 18 vs. 63 ± 17, $p < 0.02$). Homozygous women were younger than female controls (50 ± 19 vs. 63 ± 18, $p < 0.002$). No difference was observed when comparing male subjects. Women were younger than men at inclusion thrombosis (50 ± 19 vs. 65 ± 14, $p < 0.02$) and at first thrombosis (47 ± 19 vs. 64 ± 14, $p < 0.01$). Deep venous thrombosis (DVT) was seen in 33 patients (92 %), 6 (17 %) had pulmonary embolism (PE) and 3 (8 %) had combined DVT and PE. PE was less frequent in homozygous FVL women compared to female controls ($p < 0.03$). VTE recurred in 3 subjects during the duration of the study. Odds ratio for VTE in homozygous FVL patients compared to controls was 13.9 (95 % CI 9.9–19.7). We found no subjects with homozygous PTM. Double heterozygosity for FVL and PTM was seen in 12 subjects. There was no difference in age at inclusion VTE between double heterozygotes and controls (59 ± 16 vs. 63 ± 17, ns.). DVT was seen in 92 % at inclusion, 8 % had PE. Mean age at first VTE was 52 ± 17 (27–82). Consecutive homozygous FVL patients had a higher age at first thrombosis than previously described. Homozygous females are affected at an earlier age than homozygous men and female controls. It seems that thrombi in homozygous FVL have a different pattern compared to controls i.e. more prone for thrombosis in the lower extremity. The odds ratio for thrombosis among homozygous FVL seems to be lower than previously described.

Keywords Homozygous factor V Leiden · APC-resistance · Thrombosis · Venous thromboembolism · Thrombophilia · Heterozygous prothrombin mutation

Introduction

Venous thromboembolism (VTE) is a common disorder and an ever present differential diagnosis for the practicing clinician. It affects one in 1,000 individuals annually [1]. Today there are several effective methods for treating VTE and preventing VTE in patients at risk [1]. To minimize patient suffering and potential death caused by VTE it is essential that we are aware of the risk factors that predispose to the disorder, thereby identifying patients that may benefit from prophylactic treatment. Virchow [2] postulated in 1856 that the triad of changes to the vessel wall, blood composition and blood flow were involved in the formation of a thrombus. Virchow's conclusions are still valid, however, since then several underlying specific risk factors have been identified. Many of these are thoroughly investigated, thereby enabling effective

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prophylactic treatment to patients at risk of VTE. There are still a few conditions predisposing for VTE that need further documentation to be better understood [3]. Roughly a hundred years after Virchows discoveries were published Nandorff and Jordan [4] introduced the term familial thrombophilia after publishing 43 cases of inherited thrombophilia. Since then a number of different hereditary conditions have been described. The most common inherited risk factor known is the so called factor V Leiden mutation (FVL) with a prevalence in Caucasians of around 5 % [5]. This mutation leads to resistance to activated protein C, a condition first described by Dahlbäck in 1993 [6]. Subjects heterozygous for this mutation have been shown to have approximately a 5-fold risk increase of VTE compared to controls [7]. The second most common form of inherited thrombophilia is the Prothrombin G20210A mutation (PTM). The prevalence of this mutation is 1–4 % in Europeans [8], and studies have indicated a 4-fold increased risk of VTE compared to controls [7]. Less is known, however, about those homozygous for these conditions. There are only a few studies describing patients with homozygous FVL [7, 9–11]. They indicate that these patients suffer from their first thrombosis at a far younger age and have a 10- to 80-fold increased risk of VTE, compared to controls. Furthermore, individuals with homozygous FVL have been shown to have a higher rate of recurrence of VTE than controls [12]. Earlier studies have debated whether the clinical course of VTE-events in FVL patients differs from that of normal controls. For example some studies have indicated a lower frequency of pulmonary embolism (PE) in this group of patients where DVT is common. It has been hypothesized that a different structure or location of thrombi in FVL patients leads to a decreased risk of embolic events [13–15].

Another group of patients that are generally considered to have a similar risk profile of VTE as those homozygous for FVL are patients with double heterozygosity for both FVL and PTM. A meta-analysis by Emmerich et al. [7] describing 51 cases of such patients indicates that they have approximately a 20-fold risk increase, and like homozygous individuals, have their first VTE at a very young age. The purpose of this study is 2-fold. Firstly we wish to further examine the clinical features associated with occurrence of VTE in patients with homozygous FVL and PTM. Secondly, as double heterozygosity for FVL and PTM is considered to have a similar risk profile for VTE as homozygous individuals, and are generally treated in the same way, we choose to include them in the analysis.

Materials and methods

The Malmö thrombofilia study (MATS) is a prospective population based study conducted at Malmö University Hospital. This hospital is the only hospital treating VTE patients in a catchment area of approximately 280,000 people. All subjects were recruited between March of 1998 and December 2008. MATS recruited patients >18 years of age that had an objectively verified diagnosis of VTE through phlebografy, venous duplex or computed tomography. Patients were required to leave blood samples, answer a questionnaire and participate in a complete analysis of risk factors for VTE. Seventy percent of all patients treated for VTE at Malmö University Hospital were included in the study. The remaining 30 % were excluded due to unwillingness to participate, language problems, dementia or other severe illness that prevented the patient from participating. The control group constituted of all patients in the MATS database excluding the 36 patients with homozygous FVL when analysing homozygous FVL and excluding the 12 patients with double heterozygosity when analysing double heterozygosity. The control group was comparable to the control group in age and sex distribution [16]. The study was approved by the Lund University Ethical Committee and all patients provided written consent.

DNA analysis was performed using Taqman allele discrimination with gene specific assays for factor V and factor II (Applied Biosystems). Subjects homozygous for FVL, PTM and double heterozygotes were analyzed using the MATS database regarding patient age at inclusion thrombosis and patient age at first thrombosis. We also included data about level of thrombosis at inclusion VTE, total number of VTE-events in each patient, complete analysis of risk factors and heredity.

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All results are expressed as mean ± SD. Odds ratios were estimated by using data on prevalence published by Kjellberg et al. [17]. p values were calculated using Fischer's exact test and student's t test when appropriate. Results were considered significant if $p < 0.05$.

Results

In the MATS database, constituting 1,465 VTE patients, 36 (2.5 %) were found to be homozygous for FVL (21 women and 15 men). We found no patients homozygous for PTM, 12 patients (0.8 %) (6 women and 6 men) had a double mutation of heterozygous FVL and PTM. Baseline characteristics are presented in Table 1. There were no patients with protein C or S deficiency among these homozygous and compound subjects. The mean age of the 48 subjects with homozygous FVL or compound FVL and PTM was 57 ± 17 (22–91) at inclusion VTE. Women were younger than men (53 ± 20 vs. 62 ± 13, ns.). Ninety-two percent

Table 1 Baseline characteristics

	Homozygous FVL	Double heterozygosity FVL and PTM
Patient characteristics	$n = 36$	$n = 12$
Male/female sex	15/21	6/6
Ongoing VTE prophylaxis	1 (3)	0 (0)
Ongoing use of compression socks	1 (3)	1 (8)
Immobilisation	2 (6)	3 (25)
Cancer	5 (14)	0 (0)
Oral contraceptives[a]	3 (14)	0 (0)
Pregnancy[a]	3 (14)	0 (0)
Surgical intervention	2 (6)	1 (8)
Cast therapy	0 (0)	1 (8)
BMI > 30	9 (25)	3 (25)
Present tobacco use	9 (25)	2 (17)
Heredity for VTE in 1st degree relative	14 (39)	6 (50)
No known acquired risk factor	16 (44)	6 (50)

n (%)

[a] Percentage of female patients only

Table 2 Location of thrombus and age at thrombosis

Homozygous FVL	All $n = 36$	Male $n = 15$	Female $n = 21$	Controls $n = 1,429$
Clinical features				
Age at first VTE	53 ± 18	64 ± 14	47 ± 19	62 ± 17
Age at inclusion VTE	56 ± 18	65 ± 14	50 ± 19	63 ± 17
DVT	32 (89)	13 (87)	19 (90)	1,078 (75)
PE	6 (17)	4 (27)	2 (10)	435 (30)
DVT + PE	2 (6)	2 (13)	0 (0)	86 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity[a]	23 (72)	9 (69)	14 (74)	662 (61)
Distal thrombosis of the lower extremity[a]	5 (16)	3 (23)	2 (11)	300 (28)
Upper extremity	3 (9)	0 (0)	3 (16)	66 (6)
Other location or clinical diagnosis	1 (3)	1 (8)	0 (0)	50 (5)

Mean ± SD and *n* (%)

[a] The distribution of thrombi in the left lower extremity compared to the right was equal among men whereas 74 % female subjects had thrombosis of the left leg

of the patients had deep vein thrombosis (DVT) at inclusion, 15 % had PE and 6 % had combined DVT and PE. The distribution of thrombus location in the DVT group of 44 subjects was 27 % v.iliaca, 44 % v.femuropoplitea and 14 % localized to the calf. Three patients (6 %) had thrombosis of the arm (Tables 2, 3). Positive family history of VTE in first degree relatives was reported in 20 subjects (42 %). At least one additional acquired risk factor was seen in 26 subjects (54 %). At study end 21 patients (44 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. Three patients (6 %) had a total of 3 VTE-events and 3 patients (6 %) had a total of 4. Mean age at first VTE was 52 ± 18 (11–91). Women were significantly younger than men (46 ± 20 vs. 59 ± 12, $p < 0.02$).

Homozygous FVL

Patients homozygous for FVL had a mean age of 56 ± 18 (22–91) at inclusion VTE (Table 2). This was significantly younger when comparing to controls (63 ± 17, $p < 0.02$). Women were younger than men (50 ± 19 vs. 65 ± 14, $p < 0.02$). Homozygous women were younger than female controls at inclusion VTE (50 ± 19 vs. 63 ± 18, $p < 0.01$). No difference was observed when comparing male subjects (65 ± 14 vs. 63 ± 15, ns.). DVT was seen in 92 % of the patients at inclusion, 17 % had PE and 8 % had combined DVT and PE. In the female group 10 % were suffering from pulmonary embolism compared to 27 % in the male group. The distribution of thrombus location in the DVT group of 33 subjects was 33 % v.iliaca, 39 % v.femuropoplitea and 15 % localized to the calf. Three patients (9 %) had thrombosis of the arm. One subject with combined DVT and PE did not have a radiologically verified DVT, the exact localization of which thus remains unknown. The DVT diagnosis, in this patient, was set clinically after a positive PE diagnosis was verified by computed tomography. Positive family history of VTE in first degree relatives was reported in 14 subjects (39 %) (Table 1). At least one additional acquired risk factor was seen in 20 subjects (56 %). Three women were using oral contraceptives and three women were in post partum. Two patients had been immobilized >10 h. One patient had undergone major surgery prior to VTE-event. BMI > 30 was seen in nine patients. Malignant diseases had been diagnosed in five patients. One patient had cancer of the lung and died during the study period. One woman had breast cancer and one man cancer of the prostate. Two patients suffered from haematological malignancies, one of which had his inclusion VTE during warfarin treatment. At study end 13 patients (36 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far (Table 2). Two patients (6 %) had a total of 3 VTE-events and 2 patients (6 %) had a total of 4. Mean age at first VTE was 53 ± 18 (17–91). Women were significantly younger than men (47 ± 19 vs. 64 ± 14, $p < 0.01$). Odds ratio for VTE in individuals homozygous for FVL compared to controls was 13.9 (95 % CI 9.9–19.7) assuming a prevalence of 0.18 % homozygosity for FVL and 8.1 % heterozygosity for FVL in the south of Sweden as described by Kjellberg et al. [17].

Table 3 Location of thrombus and age at thrombosis

Double heterozygote FVL/PTM	All $n = 12$	Male $n = 6$	Female $n = 6$	Controls $n = 1,429$
Clinical features				
Age at first VTE	52 ± 17	53 ± 9	53 ± 24	62 ± 17
Age at inclusion VTE	59 ± 16	57 ± 9	62 ± 21	63 ± 17
DVT	11 (92)	5 (83)	6 (100)	1,099 (77)
PE	1 (8)	1 (17)	0 (0)	440 (30)
DVT + PE	0 (0)	0 (0)	0 (0)	88 (6)
Location of thrombus				
Proximal thrombosis of the lower extremity	10 (90)	5 (100)	5 (83)	662 (61)
Distal thrombosis of the lower extremity	1 (9)	0 (0)	1 (17)	300 (28)
Upper extremity	0 (0)	0 (0)	0 (0)	66 (6)
Other location or clinical diagnosis	0 (0)	0 (0)	0 (0)	50 (5)

Mean ± SD and *n* (%)

Double heterozygosity

Patients with double heterozygosity of FVL and PTM had a mean age of 59 ± 16 (30–82) at inclusion VTE (Table 3). There was no difference in age at inclusion VTE when comparing with controls (59 ± 16 vs. 63 ± 17, ns.). Mean age of women in this group was slightly higher than that of men (62 ± 21 vs. 57 ± 9, ns). DVT was seen in 92 % of the patients at inclusion, 8 % had PE while no patient had combined DVT and PE. The distribution of thrombus location in the DVT group of 11 subjects was 18 % v.iliaca, 73 % v.femuropoplitea and 9 % localized to the calf. Positive family history of VTE in first degree relatives was reported in six subjects (50 %) (Table 1). At least one additional acquired risk factor was seen in six subjects (50 %). Two patients had been immobilized >10 h. One patient had undergone major surgery prior to VTE-event. Three patients had a BMI > 30. At study end eight patients (67 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. One patient (8 %) had a total of 3 VTE-events and one patient (8 %) had a total of 4. All males had recurrent VTE (100 %), females had a recurrence rate of 33 %. Mean age at first VTE was 56 ± 16 (27–82). Women had a mean age of 60 ± 21 and men were significantly younger with a mean age of 53 ± 9 ($p < 0.03$).

Discussion

Homozygous FVL

Since first described in the mid fifties several studies have established inherited thrombophilia as a common risk factor for VTE. Still some of these conditions remain to be fully understood. Earlier studies of homozygous FVL have shed some light on the clinical features associated with this condition, however the largest studies were derived from selected materials [7, 11]. We present to our knowledge the largest cohort of consecutive patients with homozygous FVL. The mean age at inclusion thrombosis was 56 years. This was significantly younger when comparing to controls that had a mean age of 63 ($p < 0.02$). It was evident that the female subjects, with a mean age of 50, were the reason for the young age. Male subjects had a mean age of 65. When performing a gender specific comparison a significant age difference between homozygous women and female controls was revealed (50 ± 19 vs. 61 ± 20, $p < 0.02$). No such difference was observed when comparing male subjects (65 ± 14 vs. 62 ± 16, ns.). These findings indicate that women homozygous for FVL would be affected by VTE-events in a different way than males and female controls. It is well documented that oral contraceptive use and pregnancy (particularly post partum) predispose for VTE in FVL females [18–21]. We noticed a similar pattern in our material where women had two peaks of incidence (Figs. 1, 2). The first peak came at around 30 years of age and the second peak was around 60 years of age, the latter much like the male peak of incidence. Out of the nine women with thrombosis around the age of 30, six had known acquired risk factors. Three were using oral contraceptives, two were in post partum and one had ongoing pregnancy. This supports previous studies, arguing that women with known homozygous FVL should avoid oral contraceptives because of a greatly increased risk of thrombosis [19, 21]. An interesting observation is that the only women with homozygous FVL in the study that did have PE were under 30 years of age. One was pregnant with a gestational age of 23 weeks and the other was using oral contraceptives. Earlier studies have indicated that heterozygous FVL patients suffer from pulmonary embolisms to a lesser degree than controls [14, 15, 22], and it has been shown that there is no difference in frequency of PE between homozygotes and heterozygotes [11]. In our study 17 % of the subjects with homozygous FVL were suffering from PE compared to 30 % in the control group. There was no significant difference, although there was a tendency ($p < 0.051$). However, when comparing female subjects with male subjects there was a significant difference. In the group of female homozygous FVL patients 10 % were suffering from PE compared to 27 % in the male group. Our findings are consequently in accordance with previous observations that FVL patients suffer from PE to a lesser extent compared to controls but only regarding female subjects. The reason for the lower frequency of PE in FVL patients still remains undiscovered. Proximal DVT:s concerning the iliofemoral vein have been shown to cause PE

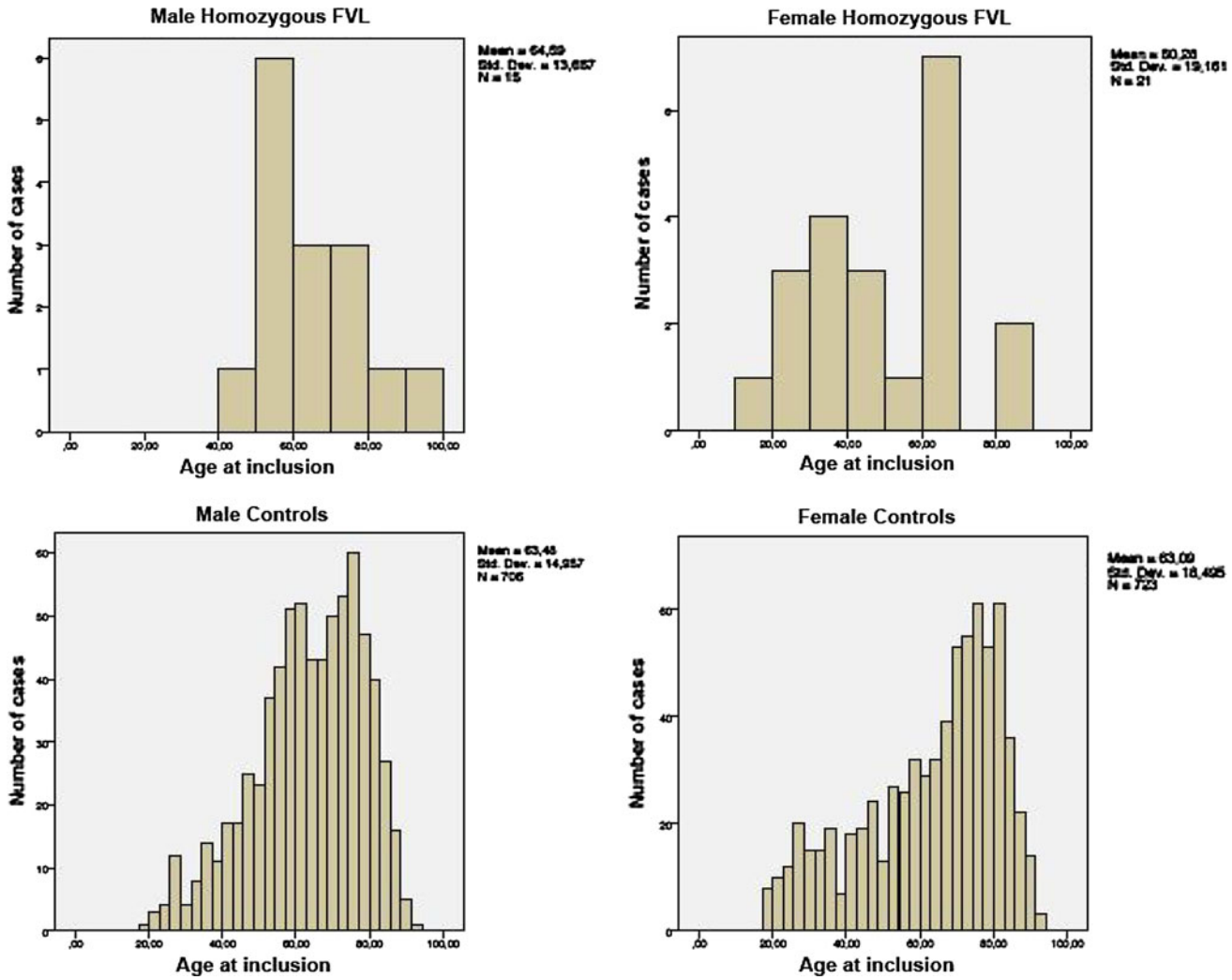

Fig. 1 Distribution of age at inclusion thrombosis

more frequently than distal DVT:s [23]. It has also been observed that thrombi in FVL patients are more distally located than thrombi in controls [13]. Combining these observations makes an appealing explanation to this phenomenon. However our results seem to contradict this theory. In our study 84 % of homozygous FVL patients had a proximal DVT (i.e. all thrombosis from the popliteal vein and above excluding PE), compared to 71 % in controls. These results are supported by similar observations made by van Stralen et al. [22]. Our findings would therefore support the theory that the thrombus in a FVL patient has a different structure, perhaps with stronger adhesive properties, thereby decreasing the risk of an embolic event. No extensive conclusions can however be drawn from our findings since only patients with symptoms of PE underwent computer tomography. At study end 13 patients (36 %) had been diagnosed with more than 1 VTE-event during their lifetime thus far. Unfortunately it was impossible to draw any conclusions from this data since a majority of patients diagnosed with homozygous FVL were given lifelong warfarin treatment. Nevertheless, the risk of recurrence after a thrombotic event is today a well described phenomena illustrated by several studies, for instance Prandoni et al. [24] where 1,626 consecutive VTE patients had a recurrence rate of 22.9 % at median 50 months' follow up without oral anticoagulation. A very recent study conducted in Malmö using the MATS database shows that patients with heterozygote FVL have an increased risk of recurrence compared to controls with an odds ratio of 2.4 [25]. However this is contradictory to the findings in an article by Willem et al. [26] showing no increased risk of recurrence compared to non thrombophilia patients. This study was not designed to look at recurrence for VTE in homozygous or double heterozygotes since several patients received lifelong treatment with anticoagulants.

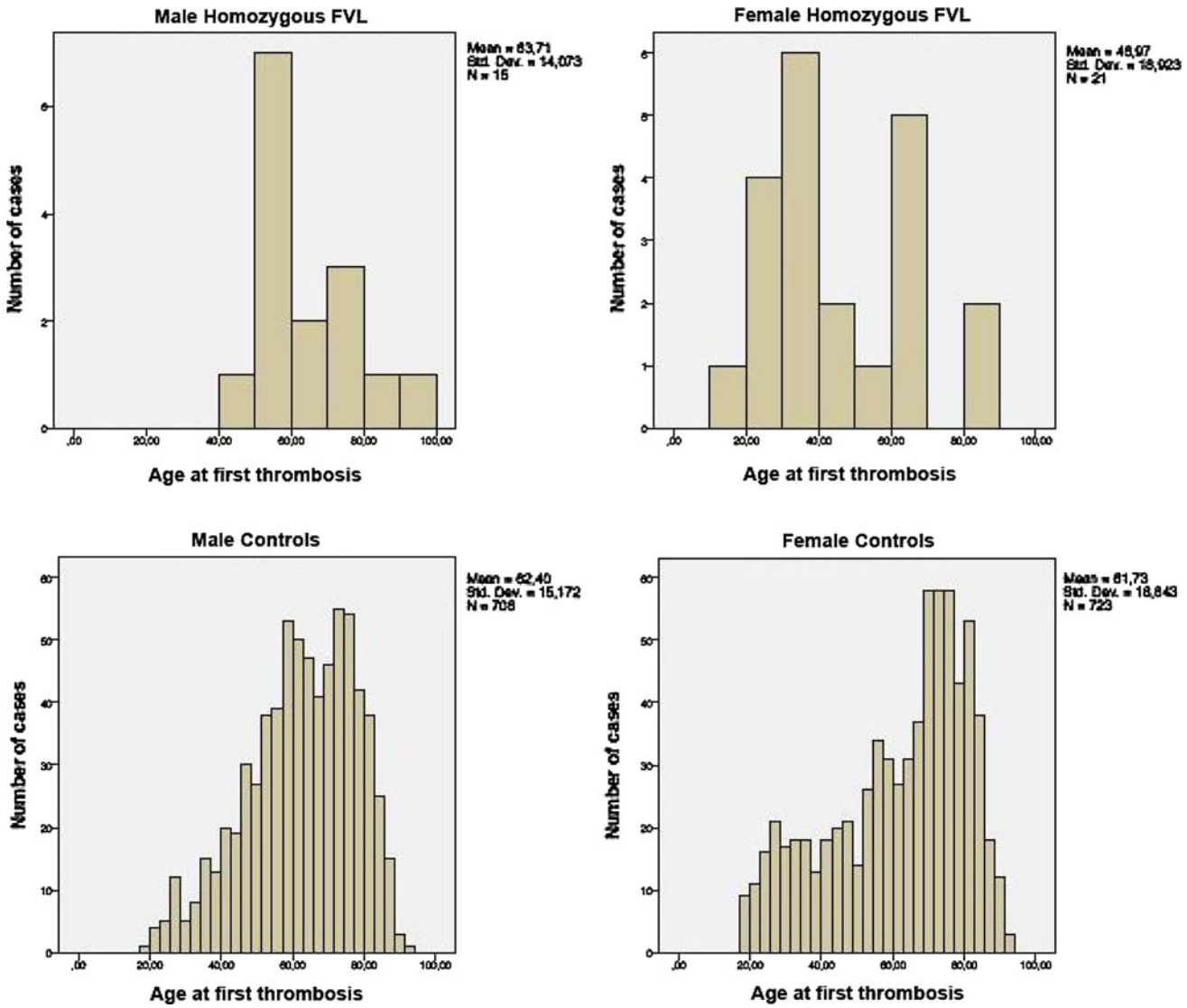

Fig. 2 Distribution of age at first thrombosis

Previous studies have indicated that patients homozygous for FVL suffer their first thrombotic event at an early age. For instance Rosendaal et al. [10] found a mean age of 31 at first thrombosis in 7 homozygous FVL patients. A large study of 85 patients with homozygous FVL conducted by the Procare group showed a mean age of 40 among men and 28 among women [11]. In our unselected population of homozygous FVL patients the mean age at first thrombosis was 64 ± 14 among men and 47 ± 19 among women (Fig. 2). In our opinion, the discrepancy in age between our study and the two aforementioned studies can be explained mainly by the fact that their materials were selected. All studies conducted on selected materials will render a young study population. We believe that our results better reflect at which age first thrombosis occurs in homozygous FVL patients. One remarkable finding is the high age at which male homozygous FVL carriers suffered their first thrombosis. In fact the homozygous FVL men were even slightly older when comparing to age at first thrombosis in male controls (64 ± 14 vs. 62 ± 15). This suggests that although males homozygous for FVL have a higher incidence of thrombosis, they resemble the average male VTE patient in terms of other clinical features. Women had a mean age of 47 ± 19 at first thrombotic event. Thus women were significantly younger than men ($p < 0.01$). As previously mentioned FVL and oral contraceptives greatly increase risk of thrombosis. Observations point to a synergistic effect when both are present [27]. More than half of the female subjects had their first VTE during fertile age, while only one male was younger than 50. Hormonal factors likely play an important role in the pathogenesis of VTE. In our study we found an odds ratio for VTE among homozygous FVL patients to be 13.9 (95 % CI 9.9–19.7) compared to non FVL carriers.

Emmerich et al. [7] showed an odds ratio of 9.85 in 30 homozygous FVL patients. These numbers stand in strong contrast to earlier observations in the LETS study where an 80-fold risk increase was reported [10]. In our material we also observed that patient age at first VTE was widely distributed (11–91). This confirms earlier observations made by Emmerich et al. [12] and suggests that factors other than genetic predisposition alone are important.

Homozygous PTM

Among the 1,480 patients in the MATS database we found no patients with homozygous PTM. This points to the rarity of this form of mutation and is confirmed by a study conducted by Rosendaal et al. [8] where 5,527 individuals, mainly Caucasians, were tested and no homozygous prothrombin mutation was found.

Double heterozygosity

The 12 subjects with double heterozygous FVL and PTM had a mean age of 52 ± 17 at first thrombosis. A meta-analysis by Emmerich et al. [7] on 51 patients with double heterozygosity reported a mean age of 34.7 at first thrombotic event. Although larger in sample size this meta-analysis consists mainly of selected materials. In contrast to the homozygous group there was no significant difference in age between female and male subjects at inclusion thrombosis and first thrombosis. There was even a tendency towards that females were older than males. One factor that might explain this is that no reports of oral contraception or pregnancies occurred in the compound group. Eight out of 12 (67 %) patients had more than one thrombotic event. There was no significant difference in age at inclusion thrombosis compared to controls (59 ± 16 vs. 63 ± 17). Recurrence rates were high, especially in males, however no definite conclusions can be drawn from this mainly because of the small sample size.

Study limitations

One of the limitations of our study is that patients <18 years of age were not included. This could potentially have meant that the mean age of inclusion thrombosis would have been somewhat lower. Another limitation is the fact that approximately 30 % of the patients that had a VTE during the study period did not participate due to reasons described in materials and methods. The figure 30 % is an estimation based on a review of hospital records of all excluded VTE patients in 1 year during the study period by a study nurse. This group matched the enrolled patients in terms of all important parameters. The sample size in this study is still too small to enable statistically stable analyses. Larger prospective studies or collaborations between centres are needed.

Conclusions

Homozygous consecutive patients had a higher age at first thrombosis than previously described. Somewhat higher risk of recurrence compared to heterozygotes and patients without thrombophilia. Homozygous females are affected at an earlier age than homozygous men and female controls. It seems that thrombi in homozygous FVL have a different pattern compared to non-thrombofilia patients, i.e. more prone for thrombosis in the lower extremity. The odds ratio for thrombosis among homozygous FVL seems to be lower than previously described. Further investigations of thrombosis in homozygous FVL are necessary.

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Conflict of interest The authors of this study report no conflict of interests.

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