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# Fat mass and obesity-associated gene rs9939609 polymorphism is a potential biomarker of recurrent venous thromboembolism in male but not in female patients

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## Abstract

Multiple genetic variations have been identified in *FTO* (fat mass and obesity-associated) gene. Among them, *FTO* rs9939609 polymorphism is shown to be associated with the risk of primary venous thromboembolism (VTE). However, its role in recurrent VTE is not known. The aim of our study was to investigate the association between *FTO* rs9939609 polymorphism and the risk of VTE recurrence in a prospective follow-up study in both male and female patients. *FTO* rs9939609 polymorphism (T/A) was analyzed in the Malmö thrombophilia study (MATS, followed for ~10 years) by using TaqMan PCR. MATS patients (n=1,050) were followed from the discontinuation of anticoagulant treatment until diagnosis of VTE recurrence or the end of follow-up. A total of 126 patients (12%) had VTE recurrence during follow-up. Cox regression analyses showed that sex modified the potential effect of *FTO* rs9939609 polymorphism on VTE recurrence. Male patients with the AA genotype for the *FTO* rs9939609 polymorphism had significantly higher risk of VTE recurrence as compared to the TT or AT genotypes (univariate hazard ratio [HR]=2.05, 95% confidence interval [CI]=1.2–3.5, p=0.009 and adjusted HR=2.03, 95% CI 1.2–3.6, p=0.013). There was no association between *FTO* rs9939609 polymorphism and VTE recurrence in female patients. In conclusion, our results show that *FTO* rs9939609 polymorphism in recurrent VTE may differ according to gender and *FTO* polymorphism may predict VTE recurrence in male patients.

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**Conflict of Interest**

Authors declare no conflict of interest.

**Keywords**

Obesity; venous thromboembolism; multivariate analysis

## Introduction

Venous thromboembolism (VTE) that includes deep vein thrombosis (DVT) and pulmonary embolism (PE) is the third most common vascular disease after coronary artery disease and stroke (1). Its consequences include recurrence, post-thrombotic syndrome, fatal PE, and severe bleeding owing to anticoagulant treatment (2). Patients who have experienced one episode of VTE are at risk of recurrent, and the risk is highest during the first 6–12 months after the first diagnosis. Around 30% of patients with primary VTE experience recurrence within 10 years (3). VTE recurrence is fatal in approximately 5–9% of cases (4). Unprovoked VTE patients (without known acquired risk factors for VTE, e.g. immobilization, trauma, major surgery, female hormone therapy, pregnancy etc.) are at higher risk of VTE recurrence than provoked VTE (5).

Standard treatment protocol for VTE patients is the use of anticoagulant therapy for 3–6 months. Prolongation of treatment time, for instance in the case of unprovoked VTE, protects patients from VTE recurrence at the cost of increased bleeding risk (6, 7). Despite several identified risk factors and prediction models such as male sex, increased D-dimers level, residual thrombosis, HERDOO2 score, Vienna prediction model and DASH score, it is still difficult to precisely predict the risk of VTE recurrence after anticoagulation therapy stops (8, 9).

Familial and twin studies have shown a substantial role of genetic factors in the development of VTE (10–12). In a recent Swedish population study, heritability of VTE was reported as 47% for male and 40% for female VTE patients (13). Despite a number of genes being identified as risk factors for VTE, a major portion of the heritability remains unknown. Moreover, results from previous studies demonstrated that many genetic risk factors for primary VTE are less important for risk prediction of recurrent VTE (14, 15). Therefore, it is important to identify new biomarkers for a better stratification of the risk of VTE recurrence in order to tailor the anti-coagulant therapy accordingly.

Obesity is the measure of body mass index (BMI) >30 kg $m^{-2}$. Being overweight or obese is a major and increasingly prevalent risk factor for multiple disorders, including VTE (16). Obesity is connected to the raised intra-abdominal pressure, decreased blood velocity in legs, inactivity, as well as prothrombotic and proinflammatory states. These factors are also known to contribute to the risk of VTE (17). Obesity is associated with almost doubling the risk of primary VTE (18). There are a lack of consistent data available on the role of BMI as a risk factor for VTE recurrence though most studies reported a slightly increased risk of VTE recurrence (19–22). Recent reports argue that BMI is not the most accurate measure of obesity and is not always feasible to measure in clinical settings. Furthermore, visceral adiposity is now suggested as more accurate measure of obesity but it needs CT/MRI that is not possible in all settings (23, 24). Therefore, the genetic factors associated with lifelong obesity are important to investigate for their role in risk prediction of VTE recurrence.

Heritability studies have provided evidence for a substantial genetic contribution (60–70%) to the obesity-related phenotypes (25, 26). Obesity is associated with a large number of common genetic variants, each with a small effect size. *FTO* (fat mass and obesity associated gene), positioned on chromosome 16, was the first common obesity susceptibility gene identified through genome-wide association studies, discovered by Frayling *et al.* (27). FTO protein is expressed in several tissues, mainly in specific parts of the muscles and brain, involved in fatty acid metabolism, energy homoeostasis, and hypothalamic regulation of food intake and appetite (27, 28). Frayling *et al.* showed that genetic variants in *FTO* gene were associated with risk of type 2 diabetes mellitus (DM). However, the primary effect was due to BMI rather than DM (27). The major contribution to this association with BMI was a cluster of 10 SNPs present in the first intron of *FTO* gene that were tightly linked to each other. This association was replicated by analyzing a single SNP (rs9939609 polymorphism) present in the first intron of *FTO* gene in 3757 type 2 diabetes patients and 5346 controls; the diabetes risk allele was significantly associated with BMI as well (27). The *FTO* gene continues to be the locus with the largest effect on obesity risk and BMI. However, the pathway whereby the rs9939609 and other *FTO* variants influence the risk of obesity remains unknown.

In a recent study, conducted by Klovaite J *et al.* in a group of 87,574 individuals of Danish descent, they found that the *FTO* rs9939609 polymorphism is significantly associated with higher risk of primary VTE (HR=1.86; 95% CI= 1.14–3.02) (29). However, the *FTO* rs9939609 variant has not been studied in recurrent VTE. The aim of the present study was to analyze the *FTO* rs9939609 variant in VTE patients and determine its possible association with VTE recurrence in both male and female patients.

## Materials and Methods

### Study subjects

The Malmö thrombophilia study (MATS) is a well-characterized cohort including 1465 VTE patients that were followed after inclusion in this study (March 1998) until VTE recurrence or death or the end of the study (December 2008) (30, 31). This study was performed at Skåne University Hospital Malmö, Sweden. At the time of inclusion, VTE events prior to the inclusion in the study, immobilization and cast therapy, location of DVT, surgical intervention, hospitalization, malignancies (past or prevalent), hormonal therapy, use of contraceptive pills, pregnancy and postpartum period (first six weeks after delivery), family history of VTE (history of VTE in first-degree relatives), and VTE recurrence during the follow-up period were recorded for all VTE patients.

The inclusion criteria in MATS were objective diagnosis of DVT and/or PE by one or more of the following methods: phlebography, computed tomography (CT), lung scintigraphy, magnetic resonance imaging (MRI) or duplex ultrasonography. MATS patients were required to answer a questionnaire and leave blood samples. The rate of consensual participation in this study was 70%. The remaining patients (30%) were excluded from the study because of the following: language problems, <18 years of age, did not participate in the blood sampling, questionnaire and complete risk factor analysis due to dementia and the presence of other severe diseases, and unwillingness to participate in the study.

Treatment of patients was performed according to the standard treatment protocol of Malmö University Hospital, i.e. initial treatment with low molecular weight heparin or unfractionated heparin and then with warfarin as an oral anticoagulant. According to hospital treatment protocol, therapy was recommended for 3–6 months for first-time VTE, with consideration of extended treatment in case of recurrent VTE. Thrombophilia was defined as presence of the factor V Leiden (FVL, rs6025) or factor II G20210A (rs1799963), or a level below the laboratory reference range of protein C (<0.7 kilo international unit (kIU)/L) or antithrombin (<0.82 kIU/L) or free protein S (female <0.5 kIU/L, male <0.65 kIU/L) in VTE patients without warfarin treatment.

Follow-up period was counted in years (Mean ± SD, 3.9 ± 2.5) after stopping the anticoagulant treatment until the diagnosis of VTE, death of the patient or the end of the study (December, 2008). The ethical committee of Lund University approved this study and all the participants provided written permission before their inclusion in the study according to the declaration of Helsinki.

## Laboratory methods

DNA was extracted from the whole blood using QiAmp 96 DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. TaqMan® SNP Genotyping Assay was used to perform genotyping of *FTO* rs9939609 polymorphism according to the manufacturer's protocol (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA). To summarize, a polymerase chain reaction (PCR) master mix (3μL) was prepared as 2.5μL Taqman master mix, 0.25μL Taqman gene-specific assay (including VIC and FAM probes for *FTO* rs9939609 polymorphism) and 0.25μL deionized water. Master Mix was then added to each well in 384 PCR plate followed by addition of 10ng genomic DNA. PCR plate (384 wells) was vortexed followed by centrifugation at 1000 rpm (revolutions per minute) for 30 seconds. For polymorphism analysis, BioRad CFX384 real-time PCR (1000 Alfred Nobel Drive Hercules, California 94547 USA) was used according to the manufacturer's instructions with the following temperature conditions, 95°C for 10 minutes followed by 40× (92°C for 15 sec, 60°C for 1min). Different alleles of *FTO* rs9939609 polymorphism were determined by using BioRad CFX manager software.

## Analysis of known thrombophilic variants

Factor II G20210A and FVL in MATS patients were analyzed by TaqMan allele discrimination assays (Applied Biosystems) as described previously (32). Protein C activities were analyzed by the chromogenic method using the Berichrom® Protein C reagent (Siemens Healthcare Diagnostics, Upplands Väsby, Sweden) (33). Analysis of free Protein S antigen concentration was performed by latex immunoassay with Coamatic® Protein S-Free (Chromogenix, Haemochrom Diagnostica AB, Gothenburg, Sweden) (34). A thrombin-based method using Berichrom Antithrombin reagent (Siemens Healthcare Diagnostics) was used for antithrombin III antigen concentration analysis (35).

## Statistical analysis

Statistical analyses were performed by using SPSS version 21 (IBM, Armonk, NY, USA). Dichotomous variables were compared by Chi-square test or Fisher's exact test, where

appropriate and continuous variables were compared by Student T-test (if data was normally distributed) or Mann-Whitney U test (if data was not normally distributed). For normally distributed variable (BMI), Mean±SD is presented whereas for skewed variable (age), median and IQR (interquartile range) is presented. Testing for effect modification between included variables was done with inclusion of interaction term. Survival curves for time to recurrent VTE by *FTO* rs9939609 genotypes are presented and log-rank test was used to compare recurrence-free survival between genotypes. Univariate and multivariate Cox regression analyses (after adjusting for BMI, family history of VTE, mild [heterozygous prothrombin G20210A or FVL] and severe thrombophilia [homozygous carriers of FVL or those patients who had natural anticoagulant deficiencies, e.g., protein C, antithrombin, protein S deficiency, and/or carriers of multiple abnormalities] and acquired risk factors for VTE) were performed using Cox proportional hazards models. For each group of patients, Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. Sensitivity analyses were performed by Multivariate Cox regression analyses including all VTE patients with an exception for those who had thrombotic events before inclusion. The follow-up period for sensitivity analyses was calculated from the time of inclusion and was adjusted for the duration of anticoagulation treatment. Hardy–Weinberg equilibrium analysis was performed to see the genotypic distribution by using web based calculator (36). To calculate the power of our results, we used online software (www.openepi.com). Linear regression analysis was performed to find the association between *FTO* rs9939609 polymorphism and BMI. *FTO* rs9939609 polymorphism has three different genotypic forms, TT (homozygous wild type), AT (heterozygous) and AA (homozygous mutated form). During data analysis, all three genotypic forms were analyzed separately as well as a recessive model for genotype analysis was also used (by combining TT+AT genotypes and compared with AA genotype).

## Results

### Clinical data of the patients

Out of 1465 VTE patients, those who had one or more thrombotic events before inclusion to this study (n=154) were excluded. Among the remaining patients (n=1311), 148 (11.3%) developed recurrent VTE during the follow-up period. In recurrent VTE patients, frequency of thrombophilia and family history of VTE was significantly higher as compared to non-recurrent VTE patients ($P<0.05$). No significant difference, however, was observed among recurrent and non-recurrent VTE patients in age, BMI, sex, DVT and PE. Table 1.

Genotypic distributions in FTO rs9939609 polymorphism did not deviate significantly in Hardy-Weinberg equilibrium analyses ($P= 0.656$).

The distribution of *FTO* rs9939609 genotypes according to sex and their association with the basic characteristics of VTE patients (age, BMI, DVT, PE and family history) are presented in Table 2. Male patients with AA genotype were at significantly higher risk of pulmonary embolism ($P=0.040$) as compared to females. No statistically significant association was found between the distribution of *FTO* genotypes and other variables including BMI in the whole study population or when associations were analyzed separately by sex (Table 2). Furthermore, no association was found between *FTO* rs9939609 polymorphism and BMI ($P>0.05$, result not shown) in linear-regression analysis.

### *FTO* rs9939609 polymorphism and risk of VTE recurrence

For the risk assessment analyses from 1311 patients, we excluded those patients who died, had VTE recurrence during anticoagulant treatment, or for whom complete information was missing (n=261). Remaining 1050 patients were followed after stopping the anticoagulant treatment and 12% (126) had VTE recurrence during the follow-up.

In the whole population, no significant association between *FTO* rs9939609 polymorphism and risk of VTE recurrence was observed (Table 3). However, in the Cox regression model, inclusion of an interaction term between *FTO* rs9939609 polymorphism and sex of the patients showed a modifying effect of sex on *FTO* rs9939609 polymorphism (*FTO* rs9939609 polymorphism *sex: HR= 0.42, 95 % CI=0.18–0.99, P=0.049). Consequently, data was stratified according to the sex and *FTO* rs9939609 polymorphism was significantly associated with higher risk of VTE recurrence in male patients (HR =1.90 and 95% CI = 1.0–3.58, P= 0.048). In multivariate analysis also (after adjusting for BMI, family history of VTE, mild and severe thrombophilia and acquired risk factors for VTE), *FTO* rs9939609 polymorphism was associated with high risk of VTE recurrence in male patients however, it didn't reach significant level when 3 genotypes were analyzed individually (HR=1.87, CI=0.95–3.54, P= 0.072). However, as T allele containing genotypes (TT and AT) has similar risk and on the Kaplan-Meier survival curve, both genotypes had similar recurrence free survival, we combined TT and AT genotypes and compared with the AA homozygous mutated genotype. Male patients that had the *FTO* rs9939609 polymorphism were at significantly higher risk of VTE recurrence in both uni- and multivariate Cox regression analyses (HR= 2.05, CI= 1.20–3.49, P= 0.009 and HR=2.03, CI= 1.16–3.55, P= 0.013 respectively) (Table 3).

To detect the power of our results, we made power calculation analyses in whole populations and male patients separately. We found that with our sample size, we could detect a risk difference as low as 1.4 and 1.9 with 95% confidence interval and 80% power in whole population and male patients respectively. Our study results showed a risk difference of 2.05 in male patients, therefore we have had enough power to detect the differences between non-recurrent VTE and recurrent VTE patients.

### *FTO* rs9939609 polymorphism and risk of VTE recurrence in unprovoked VTE patients

We also performed a sub-analysis on unprovoked first VTE patients (n=618). In this analysis, we excluded VTE patients who had a provoked first VTE (see above). A non-significant association between *FTO* polymorphism and high risk of VTE recurrence in male patients (P= 0.059, HR= 1.94, 95% CI= 0.98–3.86) was found on univariate Cox regression analysis. However, on multivariate Cox regression analysis (after adjusting our data with BMI, family history, and risk of thrombophilia), this association became statistically significant (P= 0.046, HR= 2.09, 95% CI= 1.04–4.21). (Table 4)

Survival analysis by Kaplan-Meier curve was performed to analyze whether *FTO* rs9939609 polymorphism effects recurrence-free survival. Patients having AA and TT+AT genotypes were compared and results showed a significant difference in recurrence-free survival (Figure 1A, Log-rank test, P =0.009) in male patients. Male patients having AA genotype

had significantly shorter recurrence-free survival as compared to TT and AT genotypes whereas no significant difference was observed between *FTO* genotypes and risk of VTE recurrence in female patients (Figure 1B, Log-rank test, P =0.701).

Furthermore, sensitivity analyses were performed including all MATS patients (n=1311) except those who had one or more episodes of VTE before inclusion (n=154). For sensitivity analyses, follow-up time was calculated from the time of inclusion and was adjusted for duration of anticoagulant treatment. Cox regression analyses showed that *FTO* rs9939609 polymorphism was associated with risk of VTE recurrence in male patients (P= 0.007, HR= 2.05, 95% CI= 1.21–3.46) and this association was independent of duration of anticoagulant treatment, BMI, family history of VTE, mild and severe thrombophilia and acquired risk factors for VTE (Table 1 in the Supplementary Appendix).

## Discussion

In the present follow-up study, we have investigated the role of *FTO* rs9939609 polymorphism in risk of recurrent VTE. We found that AA genotype of the *FTO* rs9939609 polymorphism was significantly associated with higher risk of VTE recurrence in male patients and this association was not attenuated after adjustment with BMI, acquired risk factors for VTE, family history, and risk of thrombophilia. To our knowledge, this is the first study in which the *FTO* rs9939609 polymorphism has been analyzed in a prospective follow-up study of VTE patients whilst taking into account the confounders that are involved in VTE recurrence.

We could not find any study investigating the role of *FTO* rs9939609 polymorphism in recurrent VTE. However, we found one Danish study in which this polymorphism was reported to be associated with high risk of primary VTE but they have not stratified the data according to the sex; therefore, it is unclear whether this polymorphism is also associated with risk of primary VTE in a sex dependent manner (29). In a prospective cohort study, Fisher *et al.* reported an association between *FTO* rs9939609 polymorphism and increased levels of C-reactive protein (CRP); a well-known inflammatory marker involved in the pathogenesis of VTE (37, 38). The mechanisms underlying the association of the *FTO* variant in the pathophysiology of VTE remain unclear. Considering the intronic location of *FTO* rs9939609 polymorphism, it can be involved in transcription of the gene as previous reports have shown (39), however, it needs to be confirmed. Another possibility could be that this polymorphism is in linkage disequilibrium with other variants in *FTO* or in neighboring obesity related genes (40). For example, this polymorphism is present close to another gene with undefined function i.e. *KIAA1005* (also known as RPGRIP1L, lies 200 base pairs away from 5' untranslated region of *FTO* gene) that opens up the possibility that genetic variation in *FTO* may affect the regulatory part of *KIAA1005* (27). However, at present, there is no obvious mechanism that explains how this intronic variant alters the function of *KIAA1005, FTO*, or any other distant genes.

Gene knockout and over expression studies in mice models have shown that FTO protein is highly expressed in the central nervous system, involved in the regulation of energy intake and metabolism (41–44). One possible explanation for the association between *FTO*

polymorphism and obesity could be that carriers of this polymorphism not only consume more food but also select the energy-dense palatable food, which suggests that *FTO* variants affect the sensing of micronutrients of the diet (45). Another suggested explanation could be the interaction of *FTO* polymorphisms with the unhealthy lifestyle factors, i.e. lack of physical activity, dietary habits and smoking that may affect the epigenetic status (DNA methylation), ultimately contributing to the development of disease (46). However, to what extent *FTO* polymorphisms interact with these factors remains to be answered in future studies.

Genetic variation in *FTO* has been the subject of several studies for a plethora of phenotypes including obesity and obesity-related phenotypes, e.g. high BMI and a significant association between *FTO* polymorphism and BMI has been previously reported (27, 29). In our study, we also analyzed *FTO* rs9939609 polymorphism for its association with BMI. However, we did not find a significant association between *FTO* rs9939609 polymorphism and BMI. As shown in previous studies, genetic factors can vary according to gender, environment and population (47–49); e.g. Factor V Leiden frequency was found to be higher in a Swedish population as compared to the populations of other geographical regions (47, 50). In agreement with our findings, Jacobsson *et al.* reported no association between *FTO* rs9939609 polymorphism and BMI in a Swedish population (51). Therefore, there is a possibility that an association between *FTO* polymorphism and BMI may be population dependent. Further studies on larger population groups are warranted to confirm the association between *FTO* rs9939609 polymorphism and BMI.

It has been reported that male patients have 2–4 fold higher risk of VTE recurrence as compared to females (52, 53). In our study, we found a marker for the risk prediction of VTE recurrence among male patients. It remains unclear why *FTO* polymorphism is associated with higher risk of disease in males only and why male patients have higher risk of VTE recurrence. Sex-specific effects of the *FTO* variants have been reported, but the importance of these effects remains unclear (54, 55). It has been reported that in female VTE patients if the cause of primary VTE is a reversible risk factor (e.g. hormone-mediated primary VTE), the risk of VTE recurrence is reduced once the hormonal exposure is removed (56). Moreover, a previous study showed that men had a higher risk of VTE recurrence if the cause of primary VTE is unprovoked (57) suggesting that male patients have persistent but still undetermined risk factors for the development of VTE and its recurrence. Our findings, i.e. male patients with *FTO* rs9939609 polymorphism have about 2 times higher risk of VTE recurrence, could at least partially contribute to the understanding of higher risk of VTE recurrence in males.

Although our study contains several strengths, including its relatively large sample size, objective diagnosis of VTE and long follow-up period, we acknowledge its limitations as well. One possible limitation of our study was the lack of data on the functional role of *FTO* rs9939609 polymorphism in VTE patients. The present study showed that *FTO* rs9939609 polymorphism was associated with the risk of VTE recurrence in male but not in female VTE patients but we do not know the potentially differential functional role of *FTO* rs9939609 polymorphism in male and female patients.

In conclusion, this is the first study in which *FTO* rs9939609 polymorphism has been studied in recurrent VTE and our results indicate that *FTO* rs9939609 polymorphism was associated with higher risk of VTE recurrence in males but not in females independent of other well-known risk factors for VTE. Further studies in other populations are needed to confirm this association as well as to examine the functional role of *FTO* rs9939609 polymorphism for VTE recurrence in male and female patients.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## List of abbreviations

| | |
|---|---|
| **A** | Adenine |
| **BMI** | Body mass index |
| **CT** | Computed tomography |
| **CI** | Confidence interval |
| **CRP** | C-reactive protein |
| **DVT** | Deep vein thrombosis |
| **DNA** | Deoxyribonucleic acid |
| ***FTO*** | fat mass and obesity-associated gene |
| **FVL** | factor V Leiden |
| **HR** | Hazard ratio |
| **kIU** | kilo international unit |
| **MRI** | Magnetic resonance imaging |
| **MATS** | Malmö thrombophilia study |
| **ng** | Nano gram |
| **PE** | pulmonary embolism |
| **rpm** | revolutions per minute |
| **SD** | Standard deviation |
| **T** | Thymine |

| | |
|---|---|
| **VTE** | venous thromboembolism |
| **μL** | Micro liter |

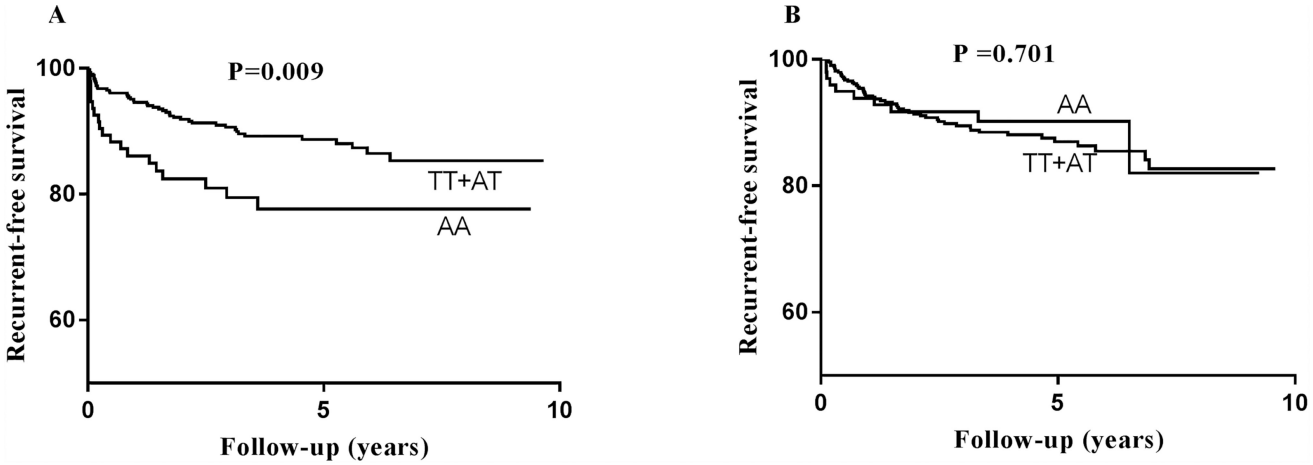


**Figure 1.**

Survival curves representing the different genotypes in *FTO* rs9939609 polymorphism and their association with risk of VTE recurrence in male (Figure 1A) and in female patients (Figure 1B). P=log-rank test.

## Table 1

Characteristics of studied population including the distribution of *FTO* rs9939609 polymorphism genotypes stratified by recurrent and non-recurrent status

| Parameters | Non recurrent VTE n (%) | Recurrent VTE n (%) | Total n (%) | ¶p-value |
|---|---|---|---|---|
| ***FTO* genotype rs9939609** | | | | |
| TT | 375 (32.5) | 44 (29.9) | 419 (32.2) | |
| AT | 575 (49.9) | 70 (47.6) | 645 (49.6) | 0.352 |
| AA | 203 (17.6) | 33 (22.4) | 236 (18.2) | |
| TT and AT | 950 (82.4) | 114 (77.6) | 1064 (81.8) | 0.172 |
| AA | 203 (17.6) | 33 (22.4) | 236 (18.2) | |
| **Age a inclusion** | | | | |
| Years, Median (IQR) | 66.4 (24) | 63.4 (24) | 65.8 (24) | 0.088† |
| **BMI** | | | | |
| Mean±SD | 26.6±4.7 | 27.4±5.1 | 26.6±4.8 | 0.066* |
| **Sex** | | | | |
| Male | 565 (48.6) | 78 (52.7) | 643 (49.0) | 0.383 |
| Female | 598 (51.4) | 70 (47.3) | 668 (51.0) | |
| **DVT+PE** | | | | |
| DVT | 736(68.2) | 98 (68.5) | 834 (68.2) | 0.323 |
| PE | 277 (25.7) | 32 (22.4) | 309 (25.3) | |
| DVT+PE | 66 (6.1) | 13 (9.1) | 79 (6.5) | |
| **Thrombophilia** | | | | |
| Yes | 390 (36.9) | 68 (49.6) | 458 (38.3) | 0.005 |
| No | 668 (63.1) | 69 (50.4) | 737 (61.7) | |
| **Family history** | | | | |
| Yes | 269 (23.5) | 47 (32.4) | 316 (24.5) | 0.024 |
| No | 875 (76.5) | 98 (67.6) | 973 (75.5) | |

DNA was not enough for genotyping in 11 samples for *FTO* rs9939609 polymorphism, DVT, deep vein thrombosis; PE, pulmonary embolism; BMI, body mass index. P-value, Chi square test until unless indicated,

*Student T-test,

†Mann-Whitney *U* test.

¶comparing non-recurrent with recurrent VTE. IQR, Interquartile range. Thrombophilia, presence of factor V Leiden, factor II G20210A, or antithrombin, protein C or free protein S levels below the laboratory reference ranges.

**Table 2**

Distribution of different genotypes of *FTO* rs rs9939609 polymorphism in studied population

| | ***FTO* genotypes** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Males (n=643)** | | **¶p value** | **Females (n=668)** | | **¶p value** | **All patients (n=1311)** | | **¶p value** |
| | **TT & AT** | **AA** | | **TT & AT** | **AA** | | **TT & AT** | **AA** | |
| **Age a inclusion** | | | | | | | | | |
| Years, Median (IQR) | 64.9 (20) | 63.7 (24) | 0.650 † | 67.5 (30) | 67.5 (28) | 0.872 † | 65.7 (24) | 65.8 (24) | 0.895 † |
| **BMI** | | | | | | | | | |
| (Mean±SD) | 26.6±4.0 | 26.9±4.2 | 0.482 * | 26.6±5.2 | 26.9±5.9 | 0.581 * | 26.6±4.7 | 26.9±5.2 | 0.402 * |
| **DVT** | | | | | | | | | |
| No | 100 (76.9) | 30 (23.1) | 0.163 | 150 (84.7) | 27 (15.3) | 0.305 | 250 (81.4) | 57 (18.6) | 0.865 |
| Yes | 420 (82.7) | 88 (17.3) | | 394 (81.2) | 91 (18.8) | | 814 (82) | 179 (18) | |
| **PE** | | | | | | | | | |
| No PE | 388 (83.4) | 77 (16.6) | 0.040 | 365 (80.9) | 86 (19.1) | 0.233 | 753 (82.2) | 163 (17.8) | 0.636 |
| PE | 132 (76.3) | 41 (23.7) | | 179 (84.8) | 32 (15.2) | | 311 (81) | 73 (19) | |
| **Thrombophilia** | | | | | | | | | |
| No | 277 (81.2) | 64 (18.8) | 0.915 | 329 (83.9) | 63 (16.1) | 0.062 | 606 (82.7) | 127 (17.3) | 0.167 |
| Yes | 194 (80.8) | 46 (19.2) | | 167 (77.7) | 48 (22.3) | | 361 (79.3) | 94 (20.7) | |
| **Family history** | | | | | | | | | |
| No | 395 (80.3) | 97 (19.7) | 0.267 | 289 (81.9) | 86 (18.1) | 0.818 | 784 (81.1) | 183 (18.9) | 0.314 |
| Yes | 116 (84.7) | 21 (15.3) | | 145 (82.9) | 30 (17.1) | | 261 (83.7) | 51 (16.3) | |

P-value, Chi square test until unless indicated, DVT, deep vein thrombosis; PE, pulmonary embolism; BMI, body mass index.

*Mann-Whitney U test,

¶comparing different genotypes of *FTO* rs9939609 rs polymorphism.

*Student T-test,

†Mann-Whitney U test.

¶comparing non-recurrent with recurrent VTE. IQR, Interquartile range. Thrombophilia, presence of factor V Leiden, factor II G20210A, or antithrombin, protein C or free protein S levels below the laboratory reference ranges.

**Table 3**

Uni- and multivariate Cox regression analyses of *FTO* rs9939609 polymorphism in recurrent VTE patients

| | All patients | | | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* |
| Genotypes | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | |
| **rs9939609** | | | | | | | | | | | | |
| TT | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| AT | 0.94 (0.63–1.41) | 0.775 | 0.86 (0.56–1.30) | 0.469 | 0.88 (0.49–1.58) | 0.674 | 0.84 (0.46–1.55) | 0.575 | 1.01 (0.58–1.77) | 0.978 | 0.89 (0.50–1.59) | 0.692 |
| AA | 1.35 (0.84–2.19) | 0.219 | 1.30 (0.80–2.13) | 0.289 | 1.90 (1.0–3.58) | 0.048 | 1.83 (0.95–3.54) | 0.072 | 0.88 (0.41–1.88) | 0.741 | 0.84 (0.39–1.79) | 0.645 |
| TT & AT | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| AA | 1.40 (0.93–2.12) | 0.111 | 1.43 (0.93–2.19) | 0.100 | 2.05 (1.20–3.49) | 0.009 | 2.03 (1.16–3.55) | 0.013 | 0.88 (0.44–1.73) | 0.701 | 0.90 (0.45–1.78) | 0.752 |

**P*** Adjusted for BMI, acquired risk factors, mild and severe thrombophilia, and family history of VTE.

**Table 4**

Uni- and multivariate Cox regression analyses of *FTO* rs9939609 polymorphism in Unprovoked VTE patients

| | All patients | | | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* |
| Genotypes | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | |
| **rs9939609** | | | | | | | | | | | | |
| TT | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| AT | 1.03 (0.63–1.70) | 0.900 | 0.85 (0.51–1.43) | 0.542 | 1.03 (0.50–2.12) | 0.939 | 0.92 (0.44–1.94) | 0.832 | 1.12 (0.56–2.32) | 0.740 | 0.90 (0.43–1.88) | 0.784 |
| AA | 1.24 (0.67–2.32) | 0.495 | 1.22 (0.65–2.27) | 0.541 | 1.97 (0.87–4.48) | 0.103 | 1.99 (0.88–4.53) | 0.101 | 0.67 (0.24–1.87) | 0.447 | 0.65 (0.23–1.82) | 0.412 |
| TT & AT | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| AA | 1.22 (0.70–2.11) | 0.478 | 1.33 (0.76–2.32) | 0.311 | 1.94 (0.98–3.86) | 0.059 | 2.09 (1.04–4.21) | 0.046 | 0.63 (0.25–1.61) | 0.333 | 0.69 (0.27–1.78) | 0.440 |

**P*** Adjusted for BMI, mild and severe thrombophilia, and family history of VTE.