Gene 647 (2018) 136–142

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

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

Abrar Ahmad[a,*], Ashfaque A. Memon[a], Jan Sundquist[a], Peter J. Svensson[b], Bengt Zöller[a], Kristina Sundquist[a,c,d]

[a] Center for Primary Health Care Research, Department of Clinical Sciences, Lund University/Skåne University Hospital, Malmö, Sweden
[b] Department of Coagulation Disorders, Skåne University Hospital, Lund University, Sweden
[c] Department of Family Medicine and Community Health, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, USA
[d] Center for Community-based Healthcare Research and Education (CoHRE), Department of Functional Pathology, School of Medicine, Shimane University, Japan

ARTICLE INFO

Keywords:
Obesity
Venous thromboembolism
Multivariate analysis

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 = 1050) 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.

1. 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 (van Schouwenburg et al., 2012). Its consequences include recurrence, post-thrombotic syndrome, fatal PE, and severe bleeding owing to anticoagulant treatment (Martinelli et al., 2014). 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 (Heit, 2015). VTE recurrence is fatal in approximately 5–9% of cases (Douketis et al., 2007). 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 (Iorio et al., 2010).

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 (Carrier et al., 2010; Kearon et al., 1999). 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

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, nanogram; PE, pulmonary embolism; rpm, revolutions per minute; SD, standard deviation; T, thymine; VTE, venous thromboembolism; µL, microliter

* Corresponding author at: Wallenberg Laboratory, 6th floor, Inga Marie Nilssons gata 53, S-20502 Malmö, Sweden.
E-mail address: abrar.ahmad@med.lu.se (A. Ahmad).

https://doi.org/10.1016/j.gene.2018.01.013
Received 30 August 2017; Accepted 3 January 2018
Available online 09 January 2018
0378-1119/

after anticoagulation therapy stops (Kyrle et al., 2010; Ensor et al., 2016).

Familial and twin studies have shown a substantial role of genetic factors in the development of VTE (Heit et al., 2004; Larsen et al., 2003; Zoller et al., 2016). In a recent Swedish population study, heritability of VTE was reported as 47% for male and 40% for female VTE patients (Zoller et al., 2017). 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 (Eichinger et al., 1999; Baglin, 2010). 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 (Lewis et al., 2009). 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 (Lorenzet et al., 2012). Obesity is associated with almost doubling the risk of primary VTE (Ageno et al., 2008). 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 (Cannegieter and van Hylckama Vlieg, 2013; Heit et al., 2001; Fahrni et al., 2015; Romualdi et al., 2007). 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 (Shah and Braverman, 2012; Shuster et al., 2012). 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 (Maes et al., 1997; Segal and Allison, 2002). 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. (Frayling et al., 2007). 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 (Frayling et al., 2007; Loos and Yeo, 2014). 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 (Frayling et al., 2007). 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 (Frayling et al., 2007). 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) (Klovaite et al., 2015). 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.

2. Materials and methods

2.1. 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) (Isma et al., 2009; Ahmad et al., 2017). 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.

2.2. 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 (3 μL) was prepared as 2.5 μL Taqman master mix, 0.25 μL Taqman gene-specific assay (including VIC and FAM probes for *FTO* rs9939609 polymorphism) and 0.25 μL deionized water. Master Mix was then added to each well in 384 PCR plate followed by addition of 10 ng genomic DNA. PCR plate (384 wells) was vortexed followed by centrifugation at 1000 rpm (revolutions per minute) for 30 s. 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 min followed by 40x (92 °C for 15 s, 60 °C for 1 min). Different alleles of *FTO* rs9939609 polymorphism were determined by using BioRad CFX manager software.