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# Polymorphisms in *PARK2* and *MRPL37* are associated with higher risk of recurrent venous thromboembolism in a sex-specific manner

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## Abstract

Recent studies indicate that mitochondrial DNA (mtDNA) dysfunction is a biomarker of oxidative stress and can predict the risk of cardiovascular diseases (CVDs). Genetic variants in *PARK2* (rs4708928) and *MRPL37* (rs10888838) genes have been shown to be associated with altered levels of mtDNA in a sex-specific manner. However, the role of these genetic variants in risk assessment of recurrent venous thromboembolism (VTE) is unknown. We investigated the role of these polymorphisms in VTE recurrence in patients from the Malmö thrombophilia study (MATS, n = 1465), followed for ~ 10 years. Genotyping was performed by TaqMan polymerase chain reaction. Female patients with *PARK2* polymorphism had significantly higher risk of VTE recurrence (Hazard ratio [HR] = 2.39, 95% confidence interval [CI] 1.09–5.24) and male patients with *MRPL37* polymorphism had a significantly higher risk of VTE recurrence (HR = 1.79, 95% CI 1.01–3.17) on multivariate Cox regression analysis. Combined analysis of these polymorphism with factor V Leiden (FVL) showed that female patients with both, FVL and *PARK2* polymorphism had even higher risk of VTE recurrence (HR = 4.49, 95% CI 1.58–12.75) compared to FVL or *PARK2* polymorphism alone or both wild-type (reference). Similarly, male patients with both FVL and *MRPL37* polymorphism had significantly higher risk of VTE recurrence (HR = 2.97, 95% CI 1.45–6.08) compared to those with FVL or *MRPL37* polymorphisms alone or the reference group. Polymorphisms in nuclear genome regulating mtDNA together with FVL may be promising biomarkers for predicting VTE recurrence in a sex specific manner. The abstract should be followed by 3-4 bullet points that highlight the major findings. The final bullet point should address future research.

**Keywords** Factor V Leiden · Genetic risk factor · Mitochondria · Predictive biomarkers · Recurrent VTE

## Highlights

- Genetic variants in *PARK2* and *MRPL37* are associated with increased risk of VTE recurrence in a sex-specific manner
- Combined analyses of *PARK2* and *MRPL37* variants with FVL increase the risk of VTE recurrence several fold in female and male patients respectively
- Our results warrant further investigation in these potentially important variants associated with VTE recurrence

## Introduction

Venous thromboembolism (VTE) comprises of deep vein thrombosis (DVT) and pulmonary embolism (PE) and is the third most frequently occurring cardiovascular disease

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(CVD) [1]. In Europe, the annual incidence of VTE is 1–2 per 1000 people with over half a million deaths annually attributed to VTE [2, 3]. The reported annual incidence rates for DVT (without PE) and PE (with or without DVT) range between 45 and 117 and 29–78 per 100,000 person-years, respectively [2]. VTE recurs frequently, and the risk of recurrence is highest during the first 6–12 months after primary VTE. Approximately 30% of patients experience VTE recurrence within 10 years of the first diagnosis. The rates of recurrent DVT have been reported as 4–13 per 100,000 person-years; 15–29 per 100,000 person-years for PE and 19–39 per 100,000 person-years for VTE. Recurrent VTE is fatal in approximately 5–9% of cases [2, 4]. The risk of recurrent VTE is higher in patients with unprovoked first VTE as compared to provoked VTE [5] (i.e. with known acquired risk factors for VTE, e.g. immobilization, trauma, major surgery, female hormone therapy, pregnancy etc.).

First VTE is normally treated with anticoagulants during a limited time period, e.g. 6 months, and recurrence may occur when anticoagulant treatment ends. However, VTE patients on continuous anticoagulant treatment can be recurrence-free, albeit at the cost of risk of severe bleeding [6]. Several risk factors and prediction models for VTE recurrence have been suggested, such as male sex, increased D-dimers level, residual thrombosis, HERDOO2 score, Vienna prediction model, and DASH score. Despite these developments, precise prediction of the risk of VTE recurrence after ending the anticoagulation treatment remains uncertain [7, 8].

VTE is a multifactorial disease that is comprised of complex interactions between genetic and non-genetic factors (environmental factors) [9]. The recognition that VTE can be caused by genetic factors dates back to the 1960s [10]. Modern methodologies estimate the heritability of VTE at around 50%, however most of it is still unknown [11, 12]. It is, therefore, clinically relevant to identify new genetic biomarkers that can identify high-risk patients for tailored anti-coagulant therapy. Moreover, previous studies have suggested different risk factors for VTE recurrence in males and females [13, 14]. Therefore, it would be equally important to identify new biomarkers that could precisely predict the risk of VTE recurrence in a sex-specific manner.

With the development of modern DNA sequencing technology, a considerable number of genes have been demonstrated to harbor genetic variations associated with the risk of cardiovascular diseases including VTE [15]. Most of these genetic risk factors are identified in the nuclear genome. Mitochondria have their own genome and contain the only non-chromosomal DNA in humans and its role in a variety of homeostatic and signaling processes is well-established [16, 17]. The mitochondrial genome contains double stranded 16.6-kb circular unmethylated DNA [18]. Mitochondrial DNA (mtDNA) is highly susceptible to oxidative stress due to its close proximity with high concentration of reactive oxidative species (ROS) produced in the mitochondrial matrix. This may lead to mitochondrial dysfunction, which is characterized by a loss of efficiency in the electron transport chain and reduction in energy production [19]. Alteration in mitochondrial function has been shown to be associated with cardiovascular diseases (CVDs) [20, 21]. Growing evidence shows that oxidative stress is the underlying mechanism involved in triggering the vascular events including atherosclerosis and thrombosis [22, 23]. However, to the best of our knowledge, its role in VTE and its recurrence is unknown.

Recently, Lopez et al. investigated the genetic mechanism controlling mtDNA function in Spanish subjects recruited from families with idiopathic thrombophilia, by using the genome-wide linkage analyses showed that 33% of alterations in mtDNA levels were due to additive effect of genes. They also reported that the genetic mechanism involved in regulation of mtDNA levels is sex-specific and found an association between rs10888838 polymorphism in mitochondrial ribosomal protein L37 (*MRPL37*), a gene involved in mitochondrial protein translation mtDNA levels, and mtDNA levels in male participants [24]. In another study, the same authors analyzed 283,437 SNPs and found a polymorphism (rs4708928) in the Parkinson protein 2 (*PARK2*) gene was significantly associated with levels of mtDNA in females [25]. In the present study we aim to investigate the potential influence of these two important genetic variants in VTE recurrence and the potential modifying role of the well-known genetic risk factor of VTE, i.e. FVL.

To the best of our knowledge, this is the first study in which genetic defects in *PARK2* and *MRPL37* genes have been analyzed in a prospective follow-up study of VTE patients.

## Materials and methods

### Study population

VTE patients from the Malmö Thrombophilia Study (MATS) were included in this study. Patients (n = 1465) were followed from time of inclusion in the study until diagnosis of VTE recurrence, death or end of the study (1998–2008). This study was performed at Skåne University Hospital Malmö, Sweden [26, 27]. Inclusion criteria in MATS include objective diagnosis for DVT and/or PE by one or more of the following methods: computed tomography (CT), lung scintigraphy, phlebography, duplex ultrasonography or magnetic resonance imaging (MRI), patients' ability to communicate in Swedish and age > 18 years. The rate of consensual participation was 70% in MATS cohort;

remaining patients (30%) were excluded because of one or more criteria, i.e. not filling in the questionnaire, language problems, the presence of other severe diseases, and in a few cases, dementia and unwillingness to participate in MATS. Information about patients was recorded regarding the following: immobilization and cast therapy, surgical intervention, hospitalization, malignancies that were diagnosed previously or at diagnosis of VTE, hormonal therapy, use of contraceptive pills, pregnancy and postpartum period (first 6 weeks after delivery), VTE events before inclusion, family history of VTE (history of VTE in first-degree relatives), VTE recurrence during follow-up period, and location of DVT.

Thrombophilia was defined as presence of the factor II G20210A (rs1799963) or factor V Leiden (FVL, rs6025) or a level below the laboratory reference range of protein C (< 0.7 kIU/L) or antithrombin (< 0.82 kIU/L) or free protein S (female < 0.5 kIU/L, male < 0.65 kIU/L) in patients diagnosed with VTE without anticoagulant treatment.

All VTE patients were treated according to the standard treatment protocol at Malmö University Hospital, i.e., low-molecular weight heparin (LMWH) or unfractionated heparin (UFH) during the initiation of oral anticoagulants (until international normalized ratio [INR] value is ≥ 2.0 but at least 5 days). Malmö University Hospital treatment protocol recommends 3–6 months of oral anticoagulant therapy for first-time VTE with the consideration of extension of treatment if VTE recurrence occurs.

Follow-up period was calculated after stopping the anticoagulant drugs (mean ± SD, 3.9 ± 2.5 years) until the diagnosis of VTE recurrence, death of the patient or end of the study (December 2008).

### DNA extraction and genotyping of SNPs

Whole blood was used to obtain genomic DNA by using QiAmp 96 DNA Blood Kit (Qiagen, Hilden, Germany) according to the supplier's recommendations. TaqMan® SNP Genotyping assays were available for both polymorphisms (*PARK2*; rs4708928 and *MRPL37*; rs10888838) with VIC and FAM probes. Genotyping was performed according to the manufacturer's protocol (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA) as reported previously [28]. FVL G1691A (rs6025) and Factor II G20210A (rs1799963) were genotyped as described previously [29]. Bio-Rad CFX manager software was used to determine various alleles of genes analyzed in this study.

### Quantification of protein C, protein S and antithrombin levels

Plasma levels of Protein C and Protein S were analyzed by using chromogenic method using the Berichrom® Protein C reagent (Siemens Healthcare Diagnostics, Upplands Väsby, Sweden) and latex immunoassay with Coamatic® Protein S-Free (Chromogenix, Haemochrom Diagnostica AB, Gothenburg, Sweden) respectively [30, 31]. Antithrombin levels were measured by a thrombin-based method using Berichrom Antithrombin (Siemens Healthcare Diagnostics) was used for [32].

### Statistical analysis

SPSS version 21 (IBM, Armonk, NY, USA) was used to perform statistical analyses. Continuous variables were compared by Mann–Whitney *U* test while dichotomous variables were compared by Chi square test or Fisher's exact test, wherever appropriate. Log-rank test was used to compare recurrence-free survival between various genotypes in *PARK2* and in *MRPL37* polymorphisms. Univariate and multivariate Cox regression analyses (after adjusting for family history of VTE, BMI, age, smoking status, thrombophilia and acquired risk factors for VTE) were performed using Cox proportional hazards models. During the data analysis, all three genotypic forms were analyzed separately as well as recessive (homozygous wild type plus heterozygous and compared with homozygous mutated form) and dominant models (homozygous wild-type compared with heterozygous plus homozygous mutated form) were used for *PARK2* and *MRPL37* polymorphisms respectively.

## Results

### Clinical data of study population

Of all the objectively diagnosed VTE patients (n = 1465), those who had one or more thrombotic events before inclusion in the study were excluded (n = 154). Among the remaining 1311 patients, 148 (11.3%) had recurrent VTE during the follow-up period. The frequency of thrombophilia was higher in recurrent VTE patients as compared to non-recurrent VTE patients (50 vs 37% respectively, P = 0.005). Regarding the patients with recurrent VTE, 32% had a family history of VTE as compared to 24% in non-recurrent VTE (P = 0.024). No significant differences were found in age, BMI, sex, smoking status, DVT and PE in recurrent and non-recurrent VTE in whole population. On stratification of data according to sex groups, we found that young male patients have high risk of VTE recurrence as compared to the older age group. In female patients, frequency of thrombophilia, patients with acquired risk factors and family history was significantly different among recurrent and non-recurrent VTE patients (Table 1).

Genotypic distribution of *PARK2* and *MRPL37* polymorphisms according to sex and their association with the basic

**Table 1** Basic characteristics of studied population including the distribution of rs4708928 and rs10888838 genotypes stratified by recurrent and non-recurrent status in male and female patients

| Parameters | All patients | | ¶P-value | Males | | ¶P-value | Females | | ¶P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Non-recurrent VTE n (%) | Recurrent VTE n (%) | | Non-recurrent VTE n (%) | Recurrent VTE n (%) | | Non-recurrent VTE n (%) | Recurrent VTE n (%) | |
| Age a inclusion | | | | | | | | | |
| Years, mean ± SD | 62.9 (17.5) | 61.3 (15.3) | 0.225* | 64.0 (15.1) | 58.5 (14.8) | **0.003*** | 61.9 (19.4) | 64.4 (15.4) | 0.221* |
| BMI | | | | | | | | | |
| Mean ± SD | 26.6 (4.7) | 27.4 (5.1) | 0.066 | 26.6 ± 3.9 | 27.0 ± 4.9 | 0.533* | 26.5 ± 5.4 | 27.7 ± 52 | 0.078* |
| DVT | | | | | | | | | |
| DVT | 886 (76) | 116 (78) | 0.608 | 445 (79) | 67 (86) | 0.177 | 441 (74) | 49 (70) | 0.568 |
| No DVT | 277 (24) | 32 (22) | | 120 (21) | 11 (14) | | 157 (26) | 21 (30) | |
| PE | | | | | | | | | |
| PE | 343 (30) | 45 (30) | 0.848 | 155 (27) | 20 (26) | 0.788 | 188 (31) | 25 (36) | 0.499 |
| No PE | 820 (70) | 103 (70) | | 410 (73) | 58 (74) | | 410 (69) | 45 (64) | |
| Thrombophilia | | | | | | | | | |
| Yes | 390 (37) | 68 (50) | **0.005** | 204 (40) | 37 (51) | 0.074 | 186 (34) | 31 (48) | **0.039** |
| No | 668 (63) | 69 (50) | | 307 (60) | 35 (49) | | 361 (66) | 34 (52) | |
| Acquired risk factors | | | | | | | | | |
| Yes | 499 (43) | 53 (36) | 0.112 | 182 (32) | 25 (32) | 0.977 | 281 (47) | 42 (60) | **0.043** |
| No | 664 (57) | 95 (64) | | 383 (68) | 53 (68) | | 317 (53) | 28 (40) | |
| Family history | | | | | | | | | |
| Yes | 269 (24) | 47 (32) | **0.024** | 121 (22) | 18 (24) | 0.768 | 148 (25) | 29 (42) | **0.004** |
| No | 875 (76) | 98 (68) | | 436 (78) | 58 (76) | | 439 (75) | 40 (58) | |
| Smoking status | | | | | | | | | |
| Never smokers | 464 (43) | 54 (40) | 0.088 | 173 (34) | 20 (29) | 0.121 | 291 (52) | 34 (50) | 0.614 |
| Former smokers | 445 (41) | 51 (37) | | 258 (50) | 30 (44) | | 187 (34) | 21 (31) | |
| Current smokers | 165 (15) | 31 (23) | | 84 (16) | 18 (26) | | 81 (14) | 13 (19) | |
| *PARK2* (rs4708928 genotype) | | | | | | | | | |
| AA | 618 (54) | 83 (57) | 0.705 | 307 (55) | 48 (61.0) | 0.141 | 311 (52) | 35 (51) | 0.785 |
| AG | 419 (36) | 52 (35) | | 204 (36) | 28 (36) | | 215 (36) | 24 (35) | |
| GG | 116 (10) | 12 (8) | | 49 (9) | 2 (3) | | 67 (11) | 10 (14) | |
| AA and AG | 1037 (90) | 135 (92) | 0.557 | 511 (91) | 76 (97) | 0.072 | 526 (89) | 59 (86) | 0.552 |
| GG | 116 (10) | 12 (8) | | 49 (9) | 2 (3) | | 67 (11) | 10 (14) | |
| *MRPL37* (rs10888838 genotype) | | | | | | | | | |
| CC | 838 (73) | 100 (68) | 0.242 | 419 (75) | 49 (63) | | 419 (71) | 51 (74) | |
| CT and TT | 315 (27) | 47 (32) | | 141 (25) | 29 (37) | **0.029** | 174 (29) | 18 (26) | 0.581 |

*DVT* deep vein thrombosis, *PE* pulmonary embolism, *BMI* body mass index

P-value, Chi square test until unless indicated, *Student T-test, ¶Comparing non-recurrent with recurrent VTE. DNA was not enough for genotyping in 11 samples for rs10888838 and rs4708928 polymorphisms respectively. Significant p-values (< 0.05) are highlighted in bold text

**Table 2** Distribution of different genotypes of rs4708928 and rs10888838 polymorphisms in studied population

| Parameters | *PARK2 (*rs4708928) | | | | | | *MRPL37* (rs10888838) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | ¶P value | Females | | ¶P value | Males | | ¶P value | Females | | ¶P value |
| | AA and AG | GG | | AA and AG | GG | | CC | CT and TT | | CC | CT and TT | |
| Age a inclusion | | | | | | | | | | | | |
| Mean ± SD | 63 (15) | 66 (13) | 0.180* | 62 (19) | 66 (17) | 0.066* | 64 (15) | 62 (15) | 0.224* | 63 (19) | 60 (18) | 0.129* |
| BMI | | | | | | | | | | | | |
| Mean ± SD | 27 ± 4 | 26 ± 4 | 0.591* | 27 ± 5 | 27 ± 6 | 0.430* | 26 ± 4 | 27 ± 4 | 0.258* | 26 ± 5 | 27 ± 6 | 0.212* |
| DVT | | | | | | | | | | | | |
| No | 121 (21) | 6 (12) | 0.145 | 151 (26) | 20 (26) | 1 | 96 (21) | 34 (20) | 0.912 | 123 (26) | 53 (28) | 0.771 |
| Yes | 447 (79) | 43 (88) | | 419 (74) | 56 (74) | | 371 (79) | 136 (80) | | 345 (74) | 138 (72) | |
| PE | | | | | | | | | | | | |
| No PE | 413 (73) | 36 (74) | 1 | 388 (68) | 53 (70) | 0.795 | 339 (73) | 125 (74) | 0.841 | 319 (68) | 130 (68) | 1 |
| PE | 155 (27) | 13 (26) | | 182 (32) | 23 (30) | | 128 (27) | 45 (26) | | 149 (32) | 61 (32) | |
| Acquired risk factors | | | | | | | | | | | | |
| Yes | 371 (68) | 17 (36) | 0.629 | 290 (53) | 32 (44) | 0.17 | 140 (31) | 63 (38) | 0.1 | 232 (52) | 98 (53) | 0.861 |
| No | 179 (32) | 30 (64) | | 257 (47) | 41 (56) | | 312 (69) | 102 (62) | | 213 (48) | 87 (47) | |
| Thrombophilia | | | | | | | | | | | | |
| Yes | 212 (42) | 16 (40) | 0.869 | 171 (34) | 25 (40) | 0.4 | 164 (40) | 69 (44) | 0.39 | 141 (35) | 59 (35) | 1 |
| No | 292 (58) | 24 (60) | | 333 (66) | 38 (60) | | 242 (60) | 86 (56) | | 267 (65) | 110 (65) | |
| Family history | | | | | | | | | | | | |
| No | 439 (78) | 37 (77) | 0.855 | 409 (73) | 54 (72) | 0.89 | 357 (78) | 134 (80) | 0.587 | 336 (73) | 136 (72) | 0.77 |
| Yes | 121 (22) | 11 (23) | | 150 (27) | 21 (28) | | 103 (22) | 34 (20) | | 122 (27) | 53 (28) | |
| Smoking status | | | | | | | | | | | | |
| Never smokers | 170 (32) | 20 (43) | 0.177 | 290 (52) | 34 (50) | 0.953 | 130 (30) | 60 (40) | 0.125 | 220 (50) | 104 (57) | 0.083 |
| Former smokers | 269 (51) | 17 (37) | | 182 (33) | 23 (34) | | 217 (51) | 69 (45) | | 157 (36) | 48 (26) | |
| Current smokers | 93 (17) | 9 (20) | | 83 (15) | 11 (16) | | 79 (19) | 23 (15) | | 64 (14) | 30 (16) | |

*DVT* deep vein thrombosis, *PE* pulmonary embolism, *BMI* body mass index

*Student T-test, ¶Comparing non-recurrent with recurrent VTE

characteristics of VTE patients (age, BMI, smoking status, DVT, PE and family history) are presented in Table 2.

Furthermore, for Cox regression analysis (see below), patients who died, had VTE recurrence during anticoagulant treatment or whom complete information about VTE recurrence was unavailable (n = 261), were excluded. Therefore, 1050 patients were followed for VTE recurrence after stopping the anticoagulant treatment and among these patients, 126 (12%) developed VTE recurrence during the follow-up period.

## *PARK2* (rs4708928) polymorphism and risk of VTE recurrence

Cox regression analyses were performed for risk assessment of VTE recurrence for *PARK2* polymorphism (known to regulate mtDNA levels in females) to investigate its association with the risk of VTE recurrence. Univariate Cox regression analysis showed that there was no significant association between various genotypes of *PARK2* polymorphism (AA, AG and GG) and risk of VTE recurrence in the whole population. Interestingly, in the Cox regression model, a modifying effect of sex on *PARK2* polymorphism was found by inclusion of an interaction term between *PARK2* polymorphism and sex (*PARK2* polymorphism*sex: HR = 6.303, CI 1.31–30.4, P = 0.022). Subsequently, we stratified patients' data according to different sex groups and a significant association between *PARK2* polymorphism and risk of VTE recurrence in female patients was found in univariate analysis (HR = 2.27, 95% CI 1.07–4.82, P = 0.033). Similar results were obtained when we adjusted our data for acquired risk factors, thrombophilia, age, BMI, smoking status and family history of VTE (HR = 2.39, 95% CI 1.09–5.24, P = 0.029). Similar results were obtained when a recessive model for genotype analysis was performed (HR = 2.31, 95% CI 1.14–4.67, P = 0.019 and HR = 2.65, 95% CI 1.26–5.59, P = 0.01 in uni- and multi-variate Cox regression analyses respectively). In the male population, however, a non-significant opposite trend was observed (Table 3).

## *MRPL37* (rs10888838) polymorphism and risk of VTE recurrence

MRPL37 polymorphism (known to regulate mtDNA in males) was analyzed for risk of VTE recurrence in the whole population by Cox regression analyses. Our results showed that CT (heterozygous) and TT (mutant) had similar effect on VTE recurrence (data not shown). Therefore, we combined T allele containing genotypes (CT and TT) and the dominant model for genotype analysis was used. Univariate Cox regression analyses showed no significant association between MRPL37 polymorphism and VTE recurrence. However, inclusion of an interaction term in the

**Table 3** Uni- and multi-variate Cox regression analyses of rs4708928 and rs10888838 polymorphisms in recurrent VTE patients

| Genotypes | All patients | | | | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* | Univariate | P | Multivariate | P* |
| | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | | HR (95% CI) | |
| rs4708928 | | | | | | | | | | | | |
| AA | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| AG | 0.78 (0.52–1.19) | 0.252 | 0.68 (0.42–1.10) | 0.114 | 0.70 (0.41–1.18) | 0.176 | 0.65 (0.36–1.18) | 0.159 | 0.96 (0.49–1.88) | 0.898 | 0.74 (0.33–1.64) | 0.458 |
| GG | 1.06 (0.57–1.97) | 0.852 | 1.26 (0.67–2.37) | 0.474 | 0.32 (0.08–1.31) | 0.112 | 0.37 (0.09–1.57) | 0.179 | 2.27 (1.07–4.82) | **0.033** | 2.39 (1.09–5.24) | **0.029** |
| AA and AG | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| GG | 1.17 (0.64–2.12) | 0.616 | 1.45 (0.78–2.67) | 0.239 | 0.36 (0.9–1.49) | 0.159 | 0.44 (0.11–1.81) | 0.255 | 2.31 (1.14–4.67) | **0.019** | 2.65 (1.26–5.59) | **0.01** |
| CC | Reference | | Reference | | Reference | | Reference | | Reference | | Reference | |
| CT and TT | 1.32 (0.89–1.96) | 0.166 | 1.35 (0.88–2.08) | 0.172 | 1.77 (1.07–2.92) | **0.026** | 1.79 (1.01–3.17) | **0.046** | 0.91 (0.48–1.73) | 0.772 | 1.0 (0.50–1.99) | 1.0 |

P* Adjusted for acquired risk factors, thrombophilia, age, BMI, smoking status and family history of VTE. Significant p-values (<0.05) are highlighted in bold text

Cox regression model showed a non-significant modifying effect of sex on MRPL37 polymorphism (MRPL37 polymorphism; sex HR = 0.513, 95% CI 0.23–1.16, P = 0.109). Stratification of data according to sex showed that *MRPL37* polymorphism is significantly associated with an increased risk of VTE recurrence in male patients only (HR = 1.77, 95% CI 1.07–2.92, P = 0.026). Similar results were found in the multivariate Cox regression analysis (HR = 1.79, 95% CI 1.01–3.17, P = 0.046) adjusted for acquired risk factors, thrombophilia, age, BMI, smoking status and family history of VTE (Table 3).

## Polymorphisms in *PARK2* and *MRPL37* and risk of VTE recurrence in unprovoked VTE patients

A sub-analysis on unprovoked first VTE patients (n = 618) showed that in these high risk patients, a non-significant trend of association between *PARK2* and *MRPL37* polymorphisms and risk of VTE recurrence was found in female and male patients respectively (HR = 2.33, 95% CI 0.98–5.52, P = 0.055 and HR = 1.65, 95% CI 0.87–3.14, P = 0.125, respectively), Supplementary Table 1.

## Combined analysis of *PARK2* or *MRPL37* polymorphisms with FVL and risk of recurrent VTE

We further analyzed these polymorphisms in combination with FVL, a well-known risk factor of VTE. Our results showed that female patients harboring both *PARK2* polymorphism and FVL had significantly higher risk of VTE recurrence as compared to FVL and *PARK2* polymorphism alone or female patients with wild type for both FVL or *PARK2*. Similarly, male patients with *MRPL37* polymorphism and FVL had significantly higher risk of VTE recurrence as compared to *MRPL37* polymorphism and FVL alone or wild type for both FVL and MRPL37. These associations remained unchanged when we adjusted our data with acquired risk factors, age, BMI, smoking status and family history of VTE (Table 4).

Similar results were found when these analyses were repeated in patients with unprovoked first VTE (Supplementary Table 2).

Kaplan–Meier curves were plotted for *PARK2* and *MRPL37* genotypes to investigate the recurrence free survival. Female patients having GG genotype in *PARK2* showed significantly shorter recurrence-free survival compared to those having AA and AG genotypes (Fig. 1b, log-rank test, P = 0.019). In contrast, no significant difference was observed between *PARK2* genotypes and risk of VTE recurrence in male patients (Fig. 1a, log-rank test, P = 0.159).

Male patients with CT and TT genotypes in MRPL37, showed significantly shorter recurrence free-survival as compared to CC genotype (Fig. 1c, log-rank test, P = 0.026). However, in female patients, there was no significant difference between *MRPL37* genotypes and recurrence-free survival (Fig. 1d, log-rank test, P = 0.772).

Moreover, we also investigated recurrence-free survival for these polymorphisms in combination with FVL by Kaplan–Meier curves. Data were stratified into four groups (FVL + *PARK2* polymorphism (GG), FVL only, *PARK2* polymorphism only and wild-type for both). Female patients together with FVL and *PARK2* polymorphism had significantly shorter recurrence-free survival as compared

**Table 4** Uni- and multi-variate Cox regression analyses of rs4708928 and rs10888838 polymorphisms in combination with FVL in recurrent VTE patients

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate HR (95% CI) | P | Multivariate HR (95% CI) | P* | Univariate HR (95% CI) | P | Multivariate HR (95% CI) | P* |
| *PARK2* (rs4708928) | | | | | | | | |
| Reference | Reference | | Reference | | Reference | | Reference | |
| rs4708928 only | 0.26 (0.04–1.89) | 0.183 | 0.28 (0.04–2.08) | 0.215 | 2.0 (0.76–5.31) | 0.163 | 2.44 (0.90–6.61) | 0.081 |
| FVL only | 1.55 (0.94–2.56) | 0.088 | 1.39 (0.80–2.42) | 0.245 | 1.81 (0.92–3.55) | 0.087 | 1.72 (0.81–3.63) | 0.157 |
| FVL + rs4708928 | 1.25 (0.17–9.14) | 0.826 | 1.48 (0.20–11.22) | 0.704 | 4.79 (1.81–12.71) | **0.002** | 4.49 (1.58–12.75) | **0.005** |
| *MRPL37* (rs10888838) | | | | | | | | |
| Reference | Reference | | Reference | | Reference | | Reference | |
| rs10888838 only | 1.27 (0.62–2.58) | 0.507 | 1.47 (0.68–3.20) | 0.33 | 1 (0.44–2.30) | 0.998 | 0.92 (0.37–2.30) | 0.866 |
| FVL only | 1.24 (0.64–2.38) | 0.52 | 1.13 (0.54–2.36) | 0.752 | 2.08 (1.04–4.19) | 0.04 | 1.78 (0.81–3.87) | 0.149 |
| FVL + rs10888838 | 3.10 (1.61–5.97) | **0.001** | 2.97 (1.45–6.08) | **0.003** | 1.67 (0.62–4.49) | 0.313 | 1.80 (0.65–5.04) | 0.259 |

P* Adjusted for acquired risk factors, age, BMI, smoking status and family history of VTE. Reference; wild type for both SNPs. Significant p-values (< 0.05) are highlighted in bold text

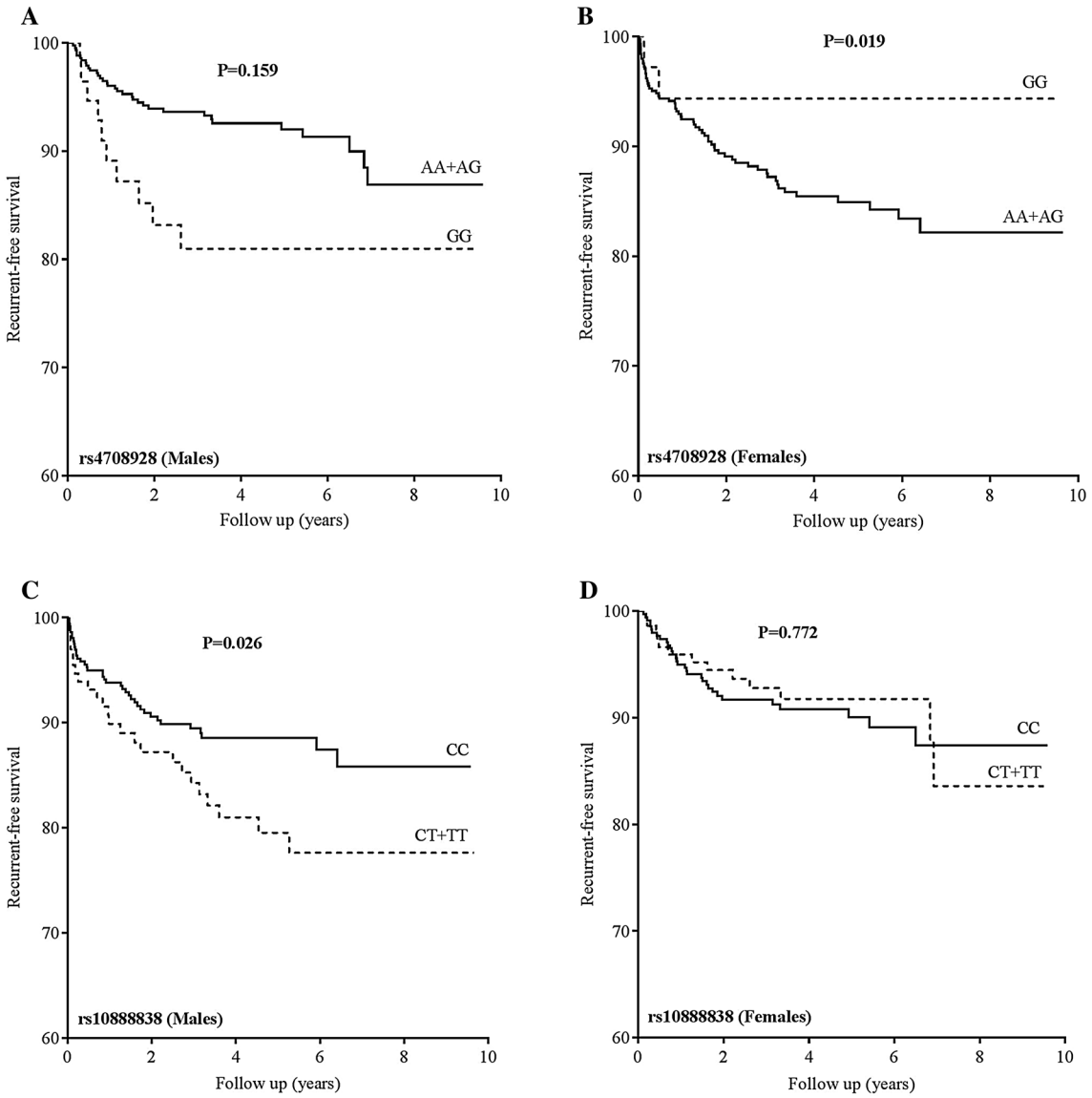


**Fig. 1** Survival curves representing the different genotypes in *PARK2* rs4708928 and *MRPL37* rs10888838 polymorphisms and their association with risk of VTE recurrence in male and in female patients. **a, b** Genotypes in rs4708928 polymorphism and their association with the risk of VTE recurrence in male (log-rank test, P = 0.159) and female patients (log-rank test, P = 0.019) respectively. **c, d** Genotypes in rs10888838 polymorphism and their association with the risk of VTE recurrence in male (log-rank test, P = 0.026) and female patients (log-rank test, P = 0.772) respectively

to females with FVL or *PARK2* polymorphism only or both wild-type (Fig. 2b, Log-rank test, P = 0.002).

Similarly, male patients having FVL and *MRPL37* polymorphism had significantly shorter recurrence-free survival as compared to males with FVL or *MRPL37* polymorphism only or both wild-types (Fig. 2a, Log-rank test, P = 0.001).

## Discussion

In the present study, we have investigated the role of two genetic variants in nuclear genome in VTE recurrence, recently suggested to regulate mtDNA levels in a sex-specific manner. Our results showed that *PARK2* polymorphism was significantly associated with a higher risk of VTE recurrence in female patients and *MRPL37* polymorphism was significantly associated with higher risk of VTE recurrence in male patients independent of acquired risk factors, thrombophilia, age, BMI, smoking status and family history of VTE. Moreover, we also showed that the risk of VTE

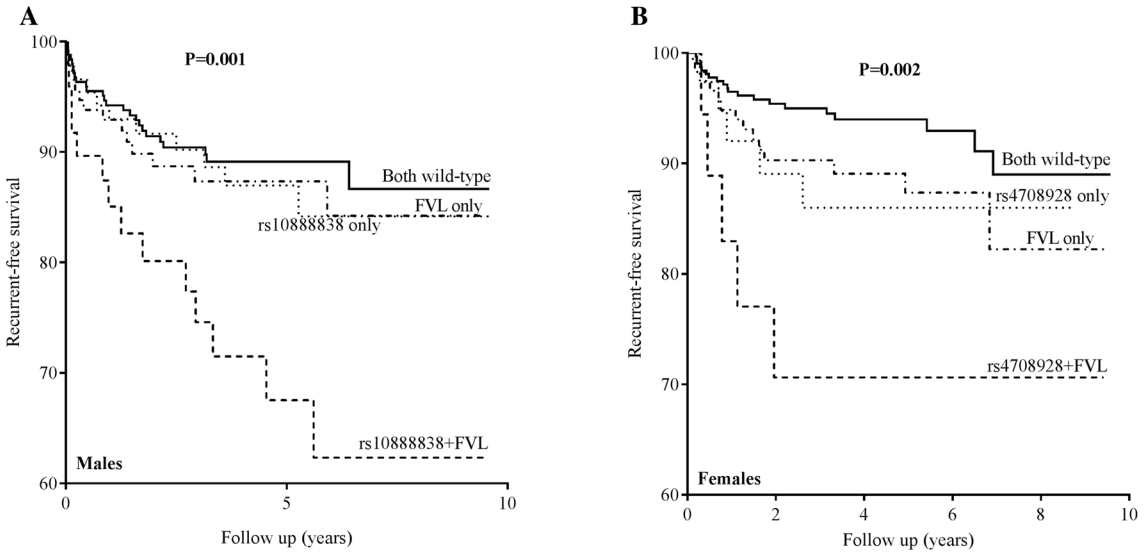


**Fig. 2** Survival curves demonstrating recurrence- free survival for various combinations of rs4708928 and rs10888838 polymorphisms with FVL. Male patients with rs10888838 polymorphism + FVL were at significantly higher risk of VTE recurrence as compared to rs10888838 polymorphism or FVL only or with no mutations (**a** log-rank test, P = 0.001). On the other hand, female patients with rs4708928 polymorphism + FVL were at significantly higher risk of VTE recurrence as compared to rs4708928 or FVL only or with no mutations (**b** log-rank test, P = 0.002)

recurrence was increased several fold (~ fivefold in females and threefold in males) when FVL was present together with *PARK2* or *MRPL37* polymorphisms in a sex-specific manner. To our knowledge, this is the first prospective follow-up study on VTE patients in which these polymorphisms have been analyzed as risk predictors of VTE recurrence.

These polymorphisms have been studied previously in other diseases including families with idiopathic thrombophilia and found to be significantly associated with the levels of mtDNA in a sex-specific manner [24, 25]. The role of mtDNA dysfunction in various cardiovascular diseases [33] signifies the importance of studying genetic variations affecting mitochondrial function in VTE. We could only find one study in which common mtDNA variants were investigated in VTE showing a weak association between some mtDNA variants and VTE risk [34]. However, it is important to mention that above study investigated common variants in mitochondrial genome in primary VTE unlike our study which includes recurrent VTE and nuclear genetic variants regulating mtDNA function.

Studies have shown that the clinical as well as genetic risk factors may differ for men and women [14, 35, 36]. Furthermore, male patients have ~ 2.6 fold higher risk of VTE recurrence as compared to females; however, underlying pathophysiology associated with this higher risk is not well understood. Moreover, men had a higher risk of VTE recurrence as compared to women if the cause of primary VTE is unprovoked [37], suggesting different genetic risk factors for men and women. In the present study, we have found that the polymorphism in *PARK2* known to regulate mtDNA function in females was associated with a higher risk of VTE recurrence in females and the polymorphism in *MRPL37* known to regulate mtDNA function in males was associated with a higher risk of VTE recurrence in males, which suggests that mitochondrial dysfunction may have a role in VTE recurrence in a sex-specific manner.

Furthermore, combined analyses of FVL (a well-known marker of VTE) and *PARK2* or *MRPL37* polymorphisms increased the risk of VTE recurrence several fold in females and males respectively. Suggesting that combined analysis of these polymorphisms and FVL may better predict VTE recurrence. Recurrent VTE is a multifactorial disease and combination of multiple genetic risk factors has been shown to better predict recurrence compared to presence of individual genetic risk factors [38]. Our results, at least partially, explain that FVL alone may not be a strong contributing factor for VTE recurrence; however, in combination with other risk factors it can better predict the risk of VTE recurrence. These results are in agreement with previous findings showing that FVL is not a strong risk factor for VTE recurrence [38, 39].

To predict the risk of VTE recurrence in unprovoked VTE patients remains a challenge. Risk of VTE recurrence is higher and can be lifelong in patients with genetic defects [29, 40]. Our results show that the risk of VTE recurrence is even higher in unprovoked VTE patients when both FVL and *PARK2* or *MRPL37* polymorphisms were present in females and males respectively. Results, however didn't reach

statistically significant level because the number of cases (recurrent VTE) in these high risk groups were smaller. Nevertheless, our results indicate that the risk of VTE recurrence, as a whole (all patients including unprovoked VTE) as well as in unprovoked VTE patients increases in the presence of *PARK2*/*MRPL37* polymorphisms together with FVL.

*MRPL37* has not been well studied for its function, while *PARK2* has a protective role in maintaining the integrity and biogenesis of mtDNA by inducing transcription and replication of mtDNA [41], thereby suggesting its role in maintaining mitochondrial function. Furthermore, VTE risk factors such as smoking, obesity and hypercholesterolemia are associated with increased mtDNA damage [42–45]. However, the role of mitochondrial dysfunction is not well studied in VTE except for a few studies suggesting an indirect role of mtDNA in venous thrombosis. For example, mitochondrial uncoupling protein-2 is linked to hyperhomocysteinemia, a risk factor for venous thrombosis [46] and higher plasma levels of mitochondrial DNA have also been associated with massive pulmonary embolism [47]. Furthermore, reactive oxygen species (ROS) are generated during mitochondrial oxidative phosphorylation that is an integral component of inflammation which is associated with pathogenesis of VTE [48]. Together, it can be speculated that the defects in genes regulating mtDNA may have a contributing role in the pathogenesis of VTE.

Though our study has several strengths including objective diagnosis of VTE, long follow-up period and its relatively large sample size, limitations of this study deserve to be mentioned. One possible limitation of our study is the lack of data on the mechanistic role of *PARK2* and *MRPL37* polymorphisms in recurrent VTE patients. However, the present findings are hypothesis generating and require further confirmation in an independent investigation.

In conclusion, we propose polymorphisms in *PARK2* and *MRPL37* as potential biomarkers for VTE recurrence in male and female patients respectively. Moreover, we demonstrate that a combined analysis of these polymorphisms together with FVL may better predict VTE recurrence.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

**Ethical approval** All procedures performed in this study, involving human participants, were in accordance with the ethical standards of the institutional research committee (Lund University) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** An informed consent was obtained from all individual participants included in the study.



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