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Brisk walking pace offsets venous thromboembolism risk equivalent to established monogenic mutations

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Abstract:
Background: Mendelian mutations in F2 and F5 genes are known risk factors for venous thromboembolism (VTE). This study aimed to explore the association between walking pace and VTE, compare its risk with Mendelian mutations, and identify if blood biomarkers mediate this effect.
Methods: We followed 445,261 UK Biobank participants free of VTE at baseline. Walking pace was self-reported, and carrier status for F2 and F5 gene mutations was determined by rs1799963 and rs6025 genotypes. We used a Cox proportional hazard model to estimate walking pace's effect on VTE risk, bidirectional Mendelian randomization (MR) analysis to assess causality, and mediation analysis to explore blood biomarkers.
Results: Over a median follow-up of 12.8 years, 11,155 incident VTE cases were identified. The 10-year incidence rates for brisk and slow walking paces were 1.32% and 3.90%, respectively. For F5 carriers, the rates were 1.70% (brisk pace) and 3.62% (slow pace). Brisk walking pace reduced VTE risk in F5 carriers (2.65%) compared to non-carriers with a slow pace (3.66%). MR analysis confirmed a causal relationship from walking pace to VTE risk. Mediation analysis revealed that serum albumin and cystatin C mediated 8.7% to 11.7% of the effect of brisk walking pace on VTE risk.
Conclusions: A slow walking pace is causally associated with increased VTE risk. A brisk walking pace mitigates VTE risk, particularly in individuals with F5 gene mutations, and this effect is partially mediated by serum albumin and cystatin C.

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Figure 1. Overall study design

Figure 2. 10-Year risk of VTE according to walking pace and mendelian mutations

Figure 3. Cumulative incidence rates of VTE according to walking pace and mendelian mutations. Cumulative incidence of VTE in the slow, steady, and brisk walking pace groups, and the non-carriers, *F2* and *F5* mutation carriers, and the combination of walking pace and mendelian mutations in both sex (**A**), female (**B**), and male (**C**). The HR and 95% CI were derived from the Cox regression model with the adjustment of age, sex, UK Biobank assessment centre, Townsend Deprivation Index, household income, smoking status, alcohol consumption, healthy diet score, physical activity**,** sedentary, duration of walking for pleasure, and frequency of walking for pleasure, body mass index, waist-to-hip ratio, hypertension history, diabetes history, and CVD history.

Figure 4. Mediation analysis.

Figure 5. Forest and radial plot of the MR analysis. The left panel shows the forest plot of causal estimates (95% confidence intervals) from different MR methods, including inverse variance weighted (IVW), simple median, weighted median, MR-Egger, maximum likelihood, and contamination mixture. The radial plot (right panel) visualizes the outlier SNPs identified in the analysis. Black dots represent valid SNPs, while magenta and cyan dots highlight SNPs identified as outliers by both IVW and MR-Egger or just by IVW, respectively. The curves depict the ratio estimate for each SNP, with blue indicating the IVW method and orange indicating the MR-Egger method.

Brisk walking pace offsets venous thromboembolism risk equivalent to established monogenic mutations

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Abstract

Background

Mendelian mutations in the Prothrombin gene (*F2*) and the factor V Leiden gene (*F5*) genes are established risk factors for venous thromboembolism (VTE). Walking pace is associated with the risk of coronary artery diseases, but no study has investigated its association with VTE. This study aimed to investigate the association and causality

between walking pace and VTE, compare its population risk with established Mendelian mutations, and determine if blood biomarkers mediate its effect.

Methods

We followed up 445,261 UK Biobank participants free of VTE at baseline. Self-reported walking pace was collected via touchscreen questionnaire at baseline. The carrier status of two Mendelian mutations in *F2* and *F5* genes was determined by the genotypes of rs1799963 (G20210A, c.*97 G > A) and rs6025 (p.R534Q), respectively. Cox proportional hazard model was used to estimate the effect of walking pace on incident VTE. We conducted a bidirectional MR analysis, by using 70 SNPs from a walking pace GWAS and 93 SNPs from a VTE GWAS as instrumental variables. We used both individual-level data and GWAS summary statistics for mediation analysis.

Results

Over a median follow-up period of 12.8 years, 11,155 incident VTE cases were identified. The 10-year incidence rates for brisk and slow walking pace were 1.32% (CI: 1.27% - 1.37%) and 3.90% (CI: 3.71% - 4.09%) respectively. For non-carriers, *F2* and *F5* carriers, the 10-year incidence rates were 1.70% (CI: 1.66% - 1.73%), 2.94% (CI: 2.66 – 3.22%) and 3.62% (CI: 3.39% - 3.84%) respectively. The overall risk of VTE for *F5* mutation carriers with a brisk walking pace (2.65%) was smaller than that for non-carriers with a slow walking pace (3.66%). For *F5* mutation carriers, brisk pace (but not steady pace) reduces the risk of VTE (*P* interaction <0.05).

Mendelian Randomization (MR) analyses displayed a causal relationship (IVW $P = 3.21\times10^{-5}$) from walking pace to VTE incidence. Mediation analysis showed that serum albumin (ALB) and cystatin C (CYS) levels partially mediated the effect of brisk walking pace on the risk of VTE incidence, with mediation proportions of 8.7% to 11.7% respectively.

Conclusions

On the population scale, the protective effect of brisk walking pace offsets the risk of VTE caused by Mendelian mutations. We provided preliminary evidence that a brisk walking pace causally reduces the risk of VTE. Serum ALB and CYS partially mediate this effect.

Keywords

walking pace; venous thromboembolism; prospective cohort; Mendelian randomization; mediation

What is known on this topic?

- Walking pace is associated with the risk of coronary artery diseases, but no study has investigated its association with venous thromboembolism (VTE).
- The extent and causality of the association between walking pace and VTE have not yet been investigated.

What does this paper add?

- A slow walking pace is associated with higher risks of VTE.
- The 10-year risk difference between brisk and slow walking pace is equivalent to that between non-carriers and carriers of the two established Mendelian mutations: G20210A (c.*97 G > A) in the the Prothrombin gene (*F2*) gene, p.R534Q in the the factor V Leiden gene (*F5*) gene.
- Through Mendelian randomization, we provided preliminary evidence that a brisk walking pace causally reduces the risk of VTE .
- Through mediation analyses, we showed that serum albumin and cystatin C levels partially mediated the effect of brisk walking pace on the risk of VTE incidence.

Introduction

Walking pace has been reported to be associated with overall cardiovascular health frailty.[1] Venous thromboembolism (VTE) is a condition encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE). It poses a significant health concern and is the third most common cardiovascular ailment in Western nations.[2] Twin research has suggested a heritability around 50%.[3, 4] It has been long recognized that rare Mendelian mutations in Prothrombin gene (*F2*) and the factor V Leiden gene (*F5*) genes contribute to inherited thrombophilia[5, 6], while recent genome-wide association studies (GWAS) and subsequent polygenic risk scores (PRS) approaches

showed that common genetic variants could also be used to identify individuals at increased risk of VTE.[6]

Risk factors for VTE include a combination of genetics and lifestyle factors such as smoking and physical inactivity.[7] Strategies for VTE prevention have shifted from primarily targeting hospital-related risk factors to promoting heart-healthy lifestyles.[6] Regular exercise is associated with a lower risk of VTE compared with no exercise.[6] However, studies reporting on the association between physical activity and VTE risk show inconsistent results[8-12]. Walking is an accessible and cost-effective form of physical activity that is widely advocated for its numerous benefits to physical, mental, and social well-being.[13, 14]

Walking pace is linked with all-cause mortality,[15] cardiovascular disease (CVD),[16] type 2 diabetes mellitus (T2DM),[17] and chronic kidney disease.[18] However, research is lacking to study how self-reported walking pace affects the incidence of VTE.

Walking pace also has a genetic cause. A GWAS on self-reported walking pace identified 70 independent associated loci.[19] These discovered genetic loci enable the Mendelian Randomization (MR) study to explore the causal role of walking pace.

Furthermore, blood metabolites serve as crucial biomarkers, offering insights into the metabolic processes underlying VTE. Recent studies have suggested that certain blood metabolites are associated with VTE risk, highlighting the potential role of metabolic health in VTE prevention. For instance, elevated levels of certain lipid

metabolites, such as glucose, creatinine, and C-reactive protein, have been linked to a higher incidence of VTE.[6]

Based on a large prospective cohort, our study aimed to first investigate whether walking pace is associated with the risk of VTE. We also examined the interaction between walking pace and genetic risk of VTE, and assessed causality through an MR study. Finally, we evaluated the role of blood biomarkers in the relationship between walking pace and VTE.

Methods

Study cohort and design

For this study, we included 469,767 participants who were self-reported of White European ancestry and had data on walking pace at baseline. The follow-up end date for this study is December 2021. After excluding 11,145 participants with baseline VTE and 13,361 without genetic data, 445,261 remained for final analysis. The overall study design is shown in **Figure 1**.

Assessment of exposure

The information on self-reported walking pace was collected from the baseline touchscreen questionnaire. All participants were asked the question, “How would you describe your usual walking pace?” Options included "slow," "steady," and "brisk," defined as <3, 3-4, and >4 miles per hour, respectively.

Case definition

Incident VTE cases were identified from hospital admission records linked to Health Episode Statistics in England and Wales and the Scottish Morbidity Records in Scotland. Incident VTE was defined as a hospital admission with an International Classification of Diseases, Tenth Revision (ICD-10) code of I80, I82, I26 and O88.2 and self-reported VTE extracted from field code 20002 (1093, 1094, 1068).[6] To further reduce the effect of comorbidities on walking pace, we sequentially excluded participants who developed VTE within the first two years of follow-up, participants with CVD or diabetes at baseline, and those with leg and chest pain, and surgery of the toe or leg. The detailed study design and procedures have been described in a previous study.[20]

Ascertainment of covariates

Covariates including age, sex, assessment centre, Townsend Deprivation Index,[21] smoking status, alcohol consumption, diet, sedentary behaviour and physical activity, were collected through touchscreen questionnaires at the baseline visit. Smoking and alcohol consumption status was self-reported as never, former, or current. Dietary factors were assessed using a previously published healthy diet score adapted according to American Heart Association guidelines.[22, 23] Physical activity was measured by International Physical Activity Questionnaires (IPAQ) group (Data-field 22032). and classified as low, moderate, or high, depending on the frequency and

intensity of walking, moderate activity, vigorous activity, and Metabolic Equivalent Task (MET)-minutes/week. Sedentary behaviour time was quantified at baseline by summing up the hours spent on TV watching, computer use (not at work), and driving. If the sum exceeded 24 h per day, total sedentary behaviour was recoded to 24 h per day.[24] Self-reported frequency (Frequency of walking for pleasure in last 4 weeks, data-Field 971) and duration (Duration walking for pleasure, data-Field 981) of walking for pleasure were collected using the baseline touchscreen questionnaire. For walking frequency, UK Biobank provided six incremental categories, along with options for “Do not know” and “Prefer not to answer”. Participants were assigned to three groups: “slow”, “average”, and “high”. “Slow” was defined as walking 1-3 times in the last 4 weeks, “average” as walking 1-3 times per week, and “high” as walking more than 3 times per week. For walking duration, UK Biobank provided seven incremental categories, along with options for “Do not know” and “Prefer not to answer”. We categorized walking duration into three groups: "short," "average," and "long." Short duration was defined as less than 30 minutes, average duration as 30 minutes to 1.5 hours, and long duration as more than 1.5 hours.**Ascertainment of Mendelian mutations**

The genotyping process of the UK Biobank study was documented elsewhere.[25] We assigned each participant into three groups: (1) non-carriers; (2) *F2* mutation carriers (G20210A mutation [rs1799963] heterozygous or homozygous); (3) *F5* mutation carriers (G1691A mutation [rs6025] heterozygous or homozygous).

Association analyses

The survival time for each participant was calculated as the duration from the time of the baseline visit to the date of diagnosis of VTE, death, loss to follow-up, or 31 December 2021, whichever occurred first. Cox proportional risk model was used to estimate the effect of walking pace on incident VTE. Hazard ratios (HRs) and 95% confidence intervals (CIs) were presented as the main metrics for association. We divided participants into three categories based on their self-reported walking pace: slow, steady, and brisk, with brisk pace as the reference group. We included up to seventy covariates in an incremental manner. That is, model 1 included five covariates, model 2 included seven more covariates, model 3 further included five more covariates. We used model 3 as the default model, unless otherwise stated. We added a model 4 to take into account the impact of missing covariates. To handle missing covariates, we employed a missing indicator for categorical variables and imputed the mean values for continuous variables.

Mendelian randomization analyses

In order to run MR between walking pace and VTE without overlapping samples, we generated a UKB-only VTE GWAS and subtracted it from the published VTE GWAS[6] meta-analysis summary statistics by using *Metasubtract*.[26] We conducted a bidirectional MR analysis, with 70 SNPs from a recently published walking pace GWAS[19], and 93 SNPs from another recently published VTE GWAS.[6] The

TwoSampleMR R package[27] was utilized to conduct MR in this study, and the inverse variance weighted (IVW) method was used for the main analysis result.

Mediation analyses

We first performed mediation analyses based on individual data using the R package "mediation". We then used IVW-product method for summary data based mediation analysis, where a total of 27 serum biomarkers from a recent GWAS study were evaluated for potential mediation roles (N = 363,228 individuals).[28]

All statistical analyses were performed using R software, version 4.3 (R Project for Statistical Computing). All tests were two sided, and $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

A total of 445,261 participants were included, with 7.4%, 52.4%, and 40.2% reporting slow, steady, and brisk walking paces, respectively (**Table 1**). The mean age was 56.7 years (SD = 8.0), and 241,282 (54.2%) were female. Participants with a slow walking pace tended to be older, reside in areas with higher Townsend Deprivation Index scores, and exhibited higher prevalence rates of hypertension, CVD, and diabetes. Additionally, slow walkers were more likely to engage in low physical activity levels, spend more time sedentary, and have higher BMI values.

Association of walking pace with VTE

During a median follow-up period of 12.8 years (5,544,739 person-years), we identified a total of 11,155 (2.50%) incident cases of VTE among a total of 445,261 participants, with 3,321 (1.85%), 6,164 (2.64%), and 1,670 (5.08%) for brisk (N=179,137), steady (N=233,223), and slow (N=32,901) walking paces, respectively. Participants with slow walking paces exhibited notably higher cumulative incidences of VTE compared to those with brisk walking pace. The association between walking pace and incident VTE is statistically significant among all four statistical models and remains significant after we excluded further samples from cases based on four exclusion criteria (log-rank $P < 0.0001$) (**Table 2)**. (**Table 2**).

Association of Mendelian mutations with VTE

Similarly, using non-carriers as a reference, the HRs for *F2* and *F5* mutation carriers were significantly higher (**Table 3)**. The associations remain significant when the number of adjusted covariates increased from five **(model 1**) to twelve (**model 2**) and further to seventeen (**model 3**). In general, the HRs for *F2* and *F5* mutation carriers increased slightly as more covariates are adjusted for. This indicates that **model 3** with seventeen covariates had a better power to detect true association signals. Based on model 3, the HR for *F2* and *F5* mutation carriers are 1.76 and 2.30 respectively.

Ten-year risk of VTE

The 10-year incidence rate for brisk, steady, and slow walking paces is 1.32%, 1.90%, and 3.90%, respectively. Similarly, the 10-year incidence rate for non-carriers, *F2*, and *F5* mutation carriers are 1.70%, 2.94%, and 3.62%, respectively. Therefore, the effect of walking pace is stronger than that of the established Mendelian mutation *F5* (a 10-year incidence rate difference of 2.58% vs. 1.92%) (**Figure 2**). There was a significant risk reduction in individuals who were non-carriers with brisk walking pace (risk = 1.24%, reduction = 2.43%) compared with those who were non-carriers but had a slow walking pace (risk = 3.66%) (**Figure 2**). Similary, among individuals with *F5* mutation carriers, the risk for those with a slow walking pace increased to 7.75%, which could be reduced to 2.65% among those with a brisk walking pace (reduction = 5.10%). Of note, the 10-year risk of VTE for both *F2* and *F5* mutation carriers with a brisk walking pace (risk:2.16% for *F2*, 2.65% for *F5*) was smaller than that for non-carriers with a slow walking pace (risk: 3.66%). The 10-year risk of VTE was significantly higher in males than in females. Males with *F5* mutation carriers and a slow walking pace had the highest 10-year risk of VTE (risk = 9.52%) (**Figure 2**).

Combined effect of walking pace and Mendelian mutations

A slow walking pace is associated with an increased cumulative incidence of VTE, even higher than the *F5* mutation carrier in all participants, with an even greater difference in females, while the opposite is true for males (**Figure 3A-C**). We found slight interaction between walking pace and genetic mutation status on VTE risk

($P_{interaction}$ <0.05). We further evaluated the joint effect of walking pace and Mendelian Mutations on the risk of incident VTE. As expected, participants with slow walking paces and *F5* mutation carriers exhibited notably higher cumulative incidences of VTE and the highest risk of VTE (HR: 3.13 [2.43-4.04]), followed by those with slow walking pace and *F2* mutation carriers (HR: 2.74 [1.88-3.99]) (**Figure 3A**). Females with slow walking pace and *F2* mutation carriers had higher cumulative incidences of VTE and the highest risk of VTE (HR: 3.33 [1.92-5.79]), while males with slow walking pace and *F5* mutation carriers had higher cumulative incidence rates and risk of VTE (HR: 3.29 [2.35-4.61]).

Mediators for associations between walking pace and risk of VTE

Given the association between walking pace and height and also between height and VTE, we first conducted a mediation analysis to assess whether slow pace is a mediator for the effect of height on VTE risk, but we found no significant causal mediation effect (P=0.8). Height is not a mediator either for the effect of slow pace on VTE risk. We next explored the mediating effect of blood biomarkers in the relationship between walking pace and risks of VTE based on indirect effect (IE), direct effect (DE), and the proportion of mediation. As shown in **Figure 4**, after adjusting for age and sex, 15 blood biomarkers partially mediated the association between walking pace and risks of VTE. Cystatin C (CYS) had the highest proportion of significant mediating effects at 10.4%, followed by albumin (ALB) at 8.8%.

Mendelian Randomization Analysis

A total of 63 overlapping SNPs were included as instrumental variables for the MR analysis. The IVW method *P*-value was 3.21×10^{-5}. This significance is supported by four other commonly used testing methods: simple median, weighted median, maximum likelihood, and contamination mixture (**Figure 5**). Consistent with our core MR methods, the IVW radial MR causal estimates suggested a significant causal association between brisk walking pace and reduced risk of VTE ($P = 3.14 \times 10^{-5}$). The plot indicates several outliers that deviate from the overall causal relationship between walking pace and VTE (**Figure 5)**. Further analysis using MR-PRESSO identified several SNPs as outliers, which were subsequently corrected. After adjusting for these outliers, the causal estimate between walking pace and VTE became even stronger, with an outlier-corrected *P*-value of 6.21×10^{-10}. Additionally, the MR-Egger intercept test yielded *P*-values exceeding 0.05 ($P = 0.787$), indicating the absence of horizontal pleiotropy-induced effects on the MR results. In reverse MR Analysis, no causal effect of VTE on walking pace was observed (IVW method $P = 0.05619$). After a series of processes for exploring exposure-blood biomarkers-outcome causal pathways. There are mediating effects of ALB (mediation proportion of 11.7%, $P = 0.0180$) and CYS (mediation proportion of 8.7%, $P = 0.0037$) in the causal relationship between walking pace and VTE (**Figure 4**).

Discussion

We examined the association between walking pace, genetic predisposition, and the risk of incident VTE in a large prospective cohort. Our study demonstrates a significant relationship between walking pace and the risk of venous thromboembolism (VTE), with slow walking pace associated with a markedly increased risk. Through Mendelian Randomization, we offered preliminary evidence on the causality of brisk walking pace on reduced risk of VTE. Both CYS and ALB are significant mediators in the relationship between walking pace and VTE risk. These findings have substantial implications for public health and clinical practice, as they highlight the potential of walking pace as a modifiable risk factor in the prevention of VTE. To the best of our knowledge, this is the first large-scale study examining the association between walking pace and VTE risk. Our observations were consistent with existing evidence supporting the positive effects of brisk walking pace on coronary artery disease[29], stroke[30], T2DM[17], and all-cause mortality.[31]

The prospective design, large sample size, long-term follow-up (mean 12.8 years), and high-quality data from the UK Biobank allowed us to consider the abundance of information on sociodemographic factors, lifestyles, and other covariates, providing sufficient statistical power. Our study extends the current understanding of VTE risk factors by incorporating physical capability, specifically walking pace, into the risk assessment framework. Previous studies have primarily focused on genetic predispositions and traditional lifestyle factors such as diet, smoking, and overall

physical activity levels. The novel finding that walking pace itself can influence VTE risk underscores the importance of considering specific physical behaviours in VTE prevention strategies.

The findings of this study have direct clinical implications. Healthcare providers should consider incorporating assessments of walking pace into routine evaluations of patients, particularly those with known risk factors for VTE. Interventions aimed at promoting brisk walking could be a simple yet effective strategy to reduce VTE risk. Such interventions could be particularly beneficial for individuals with genetic predispositions to VTE, as our study suggests that brisk walking can mitigate the elevated risk associated with *F2* and *F5* mutations. From a public health perspective, promoting walking at a brisk pace could serve as a cost-effective and widely accessible intervention to reduce VTE incidence at the population level. Public health campaigns and community programs designed to encourage brisk walking could have a significant impact on reducing the overall burden of VTE. Additionally, urban planning that promotes walkable environments and provides safe, accessible spaces for walking could further support these efforts.

Brisk walking pace enhances aerobic fitness,[32] and is strongly correlated with maximal oxygen uptake.[33] Recently, studies have shown that blood metabolites are key indicators of the metabolic processes related to VTE. Therefore, we further investigated the mediating role of blood biomarkers in the influence of walking pace on VTE. The results show that CYS and ALB play a partially mediating role in VTE

induced by walking pace. CYS plays a crucial role in the anti-oxidative processes.[34, 35] An imbalance in CYS level is associated with cardiovascular disorders.[36] Albumin is the most abundant plasma protein and plays a crucial role in maintaining redox homeostasis. It contains multiple thiol groups, primarily in the form of cysteine residues, which can undergo reversible oxidation and reduction.[35] Albumin exhibits antioxidant, antiplatelet, and antithrombotic activities.[37] It also binds antithrombin, inhibits liver synthesis, and enhances the neutralization of some coagulation factors .[38, 39] Oxidative stress has been linked to VTE through the oxidation of fibrinogen.[40] This insight into the role of blood biomarkers enhances our understanding of the biological mechanisms linking physical activity and VTE, and underscores the importance of metabolic health in VTE prevention.

Despite the robust findings, our study has several limitations. The self-reported nature of walking pace introduces the possibility of reporting bias. Although we carefully considered adjustments for potential confounding factors, the effects of residual confounding or unmeasured factors may still exist. In the current direct oral anticoagulant era, only the sickest patients are hospitalized. Therefore, the control group in our study might still contain individuals who could be diagnosed as cases. Future studies that utilize more sensitive approaches to identify cases might increase statistical power.

The biological mechanisms underlying the relationship between slow walking pace and increased VTE risk are multifaceted. Slow walking pace may reflect underlying

frailty, reduced muscle strength, and poorer cardiovascular and metabolic health, all of which are known risk factors for VTE. Additionally, slow walkers may engage in less overall physical activity, leading to prolonged periods of immobility, which is a well-established risk factor for VTE due to venous stasis. Furthermore, the inflammatory and coagulation pathways could be influenced by physical activity levels. Regular brisk walking has been shown to improve endothelial function, reduce inflammation, and enhance fibrinolytic activity, all of which contribute to a reduced risk of thrombus formation. In contrast, slow walking pace may be indicative of reduced engagement in these beneficial activities, thereby increasing VTE risk. Finally, the study population, primarily of European descent, may limit the generalizability of the findings to other ethnic groups.

Conclusions

In this population-based study of adults in the UK, a slow walking pace is associated with higher risks of VTE, especially among participants with slow walking paces and carriers with Mendelian mutation in the Prothrombin gene (*F2*) and the factor V Leiden gene (*F5*). We provided preliminary evidence that a brisk walking pace causally reduces the risk of VTE. Both CYS and ALB are significant mediators in the relationship between walking pace and VTE risk. Slow walking pace is a promising modifiable risk factor to consider for lifestyle interventions and may be a potential

target for the treatment or prevention of VTE. Future research should aim to replicate these findings in diverse populations and explore the mechanisms through which walking pace influences VTE risk in greater detail.

Ethics approval

All participants provided written informed consent at their baseline visit. Ethical approval of the UK Biobank study was obtained from the National Information Governance Board for Health and Social Care and the National Health Service North West Multi-Centre Research Ethics Committee (Ref 11/NW/0382). This research was conducted using the UK Biobank resources under application 66137.

Author contributions

All authors meet authorship criteria by contributing to components of research conception, design, interpretation of results, and manuscript revisions. JH., W.X. conceptualized and designed the study, conducted formal data analyses, and drafted the initial manuscript. C.Y., A.S.P contributed to data analysis and manuscript writing. Y.Q., R.S., J.M.R and V.N. provided input into the study design and revised the manuscript. T.W., PWC.L. interpreted the results, and revised the manuscript. All authors reviewed the final manuscript as submitted.

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Conflict of interest

None declared.

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Table 1. Baseline characteristics of participants by walking pace group

Characteristics	Walking pace		
	Brisk	**Steady**	**Slow**
N	179,137	233,223	32,901
Age (years)	55.3 (8.1)	57.4 (7.9)	59.3 (7.2)
Sex, male (%)	46.4	45.5	45.0
Townsend Deprivation Index	-1.7 (2.8)	-1.5 (3.0)	-0.2 (3.5)
Physical activity (%)			
low	13.0	19.5	44.3
moderate	40.2	42.2	36.4
high	46.8	38.3	19.4
Sedentary (h/day)	4.1 (2.4)	4.6 (2.5)	5.4 (3.0)
Duration of walking for pleasure			
short	14.7	17.2	17.5
average	44	38.2	18.1
long	41.3	44.6	64.5
Frequency of walking for pleasure			
low	27.1	26.9	15.6
average	32.7	27.8	14.5
high	40.3	45.3	69.9
Body mass index (kg/m^2)	25.8 (3.8)	28.0 (4.6)	31.2 (6.5)
Waist-to-hip ratio	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Hypertension history (%)	48.8	57.9	66.5
CVD history (%)	13.0	19.7	40.0
Diabetes history (%)	4.5	9.3	24.1

* Continuous and categorical variables are presented as means (SDs) and percentages (%), respectively

Table 2. Association between walking pace and incident VTE

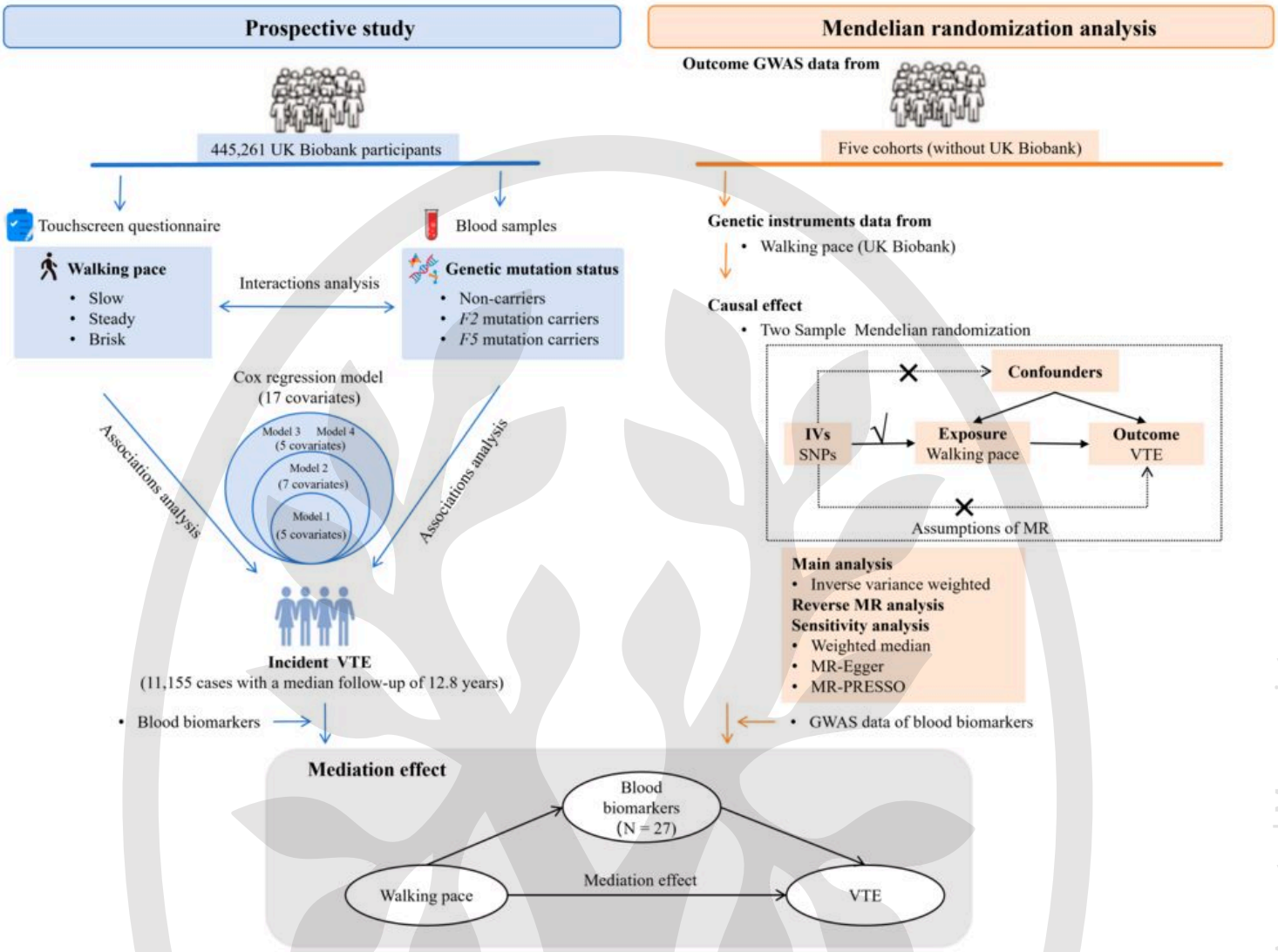
	N	Brisk	Steady	Slow	*P* trend
Model (incrementally adjusting for more covariates)					
Model 1	445,261	1.00	1.28 (1.22-1.34)	2.30 (2.15-2.46)	<0.0001
Model 2	445,261	1.00	1.25 (1.19-1.32)	2.11 (1.94-2.31)	<0.0001
Model 3	445,261	1.00	1.13 (1.06-1.19)	1.52 (1.38-1.66)	<0.0001
Model 4	445,261	1.00	1.10 (1.05-1.15)	1.43 (1.34-1.54)	<0.0001
Exclusion of subjects with baseline condition					
VTE *	441,643	1.00	1.12 (1.05-1.19)	1.45 (1.31-1.60)	<0.0001
CVD	422,184	1.00	1.11 (1.05-1.18)	1.55 (1.40-1.71)	<0.0001
T2DM	416,458	1.00	1.11 (1.04-1.17)	1.62 (1.47-1.80)	<0.0001
Leg/toe Dx#	378,589	1.00	1.07 (1.01-1.14)	1.61 (1.43-1.81)	<0.0001

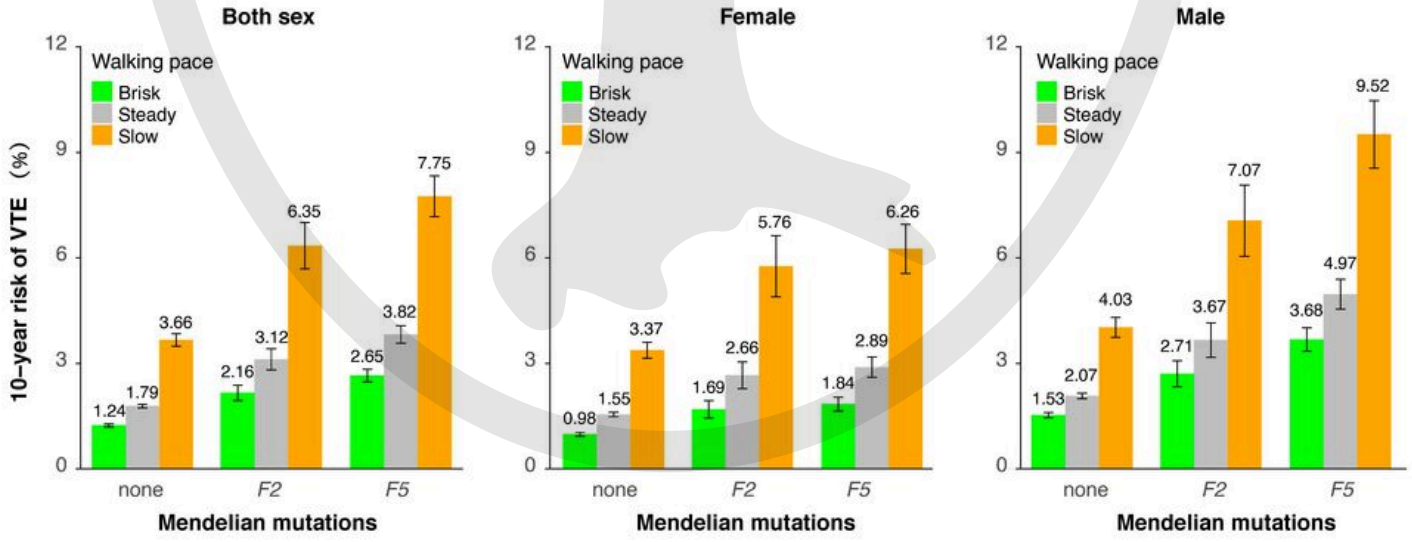
Model 1 adjusted for age (continuous, years), sex (male, female), UK Biobank assessment centre, Townsend Deprivation Index (continuous), and household income (less than £18,000, £18,000 to £29,999, £30,000 to £51,999, £52,000 to £100,000, and greater than £100,000). **Model 2** adjusted for all covariates used in Model 1, plus smoking status (current, previous, never), alcohol consumption (current, previous, never), healthy diet score (0, 1, 2, 3, 4, 5), physical activity (low, moderate, high), sedentary (continuous, hour/day), duration of walking for pleasure (short, average, long), and frequency of walking for pleasure (slow, average, high). **Model 3** adjusted for all covariates used in Model 2, plus body mass index (continuous, kg/m^2), waist-to-hip ratio (continuous), hypertension history (yes/no), diabetes history (yes/no), and CVD history(yes/no). **Model 4** adjusted for Model 3 and imputed variables. * removed subjects with VTE diagnosed within 2 years from baseline; # removed subjects with T2DM; # removed subjects with chest pain, surgery of toe or leg.

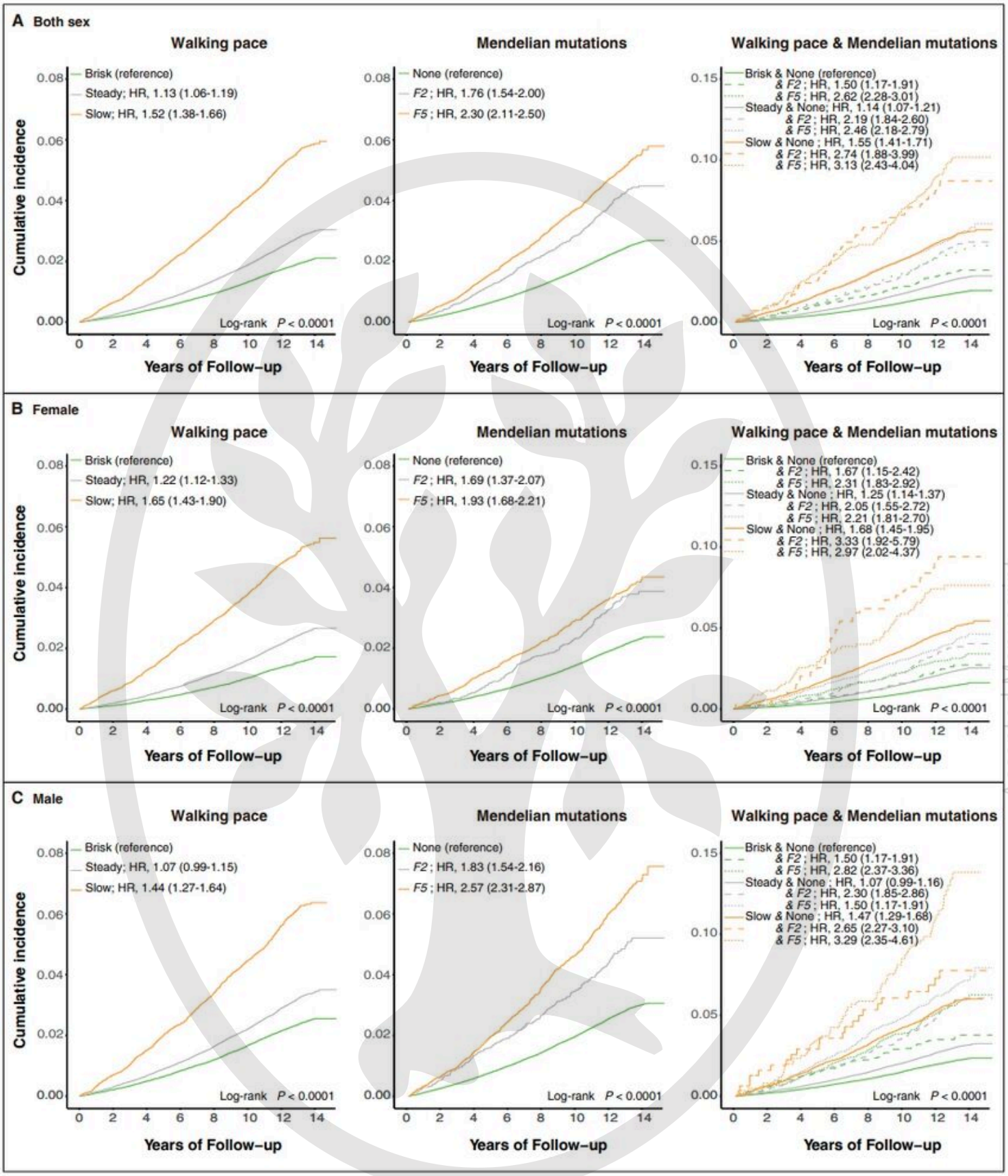
Table 3. Association between Mendelian mutations and incident VTE

	Mendelian mutations			
	Non-carriers	***F2*** **mutation carriers**	***F5*** **mutation carriers**	***P*** **trend**
Cases/N	9,770/415,589	409/10,062	976/19,610	
Model 1	1.00	1.75 (1.57-1.95)	2.25 (2.09-2.42)	<0.0001
Model 2	1.00	1.77 (1.56-2.02)	2.28 (2.09-2.48)	<0.0001
Model 3	1.00	1.76 (1.54-2.00)	2.30 (2.11-2.50)	<0.0001
Model 4	1.00	1.78 (1.61-1.97)	2.22 (2.08-2.37)	<0.0001

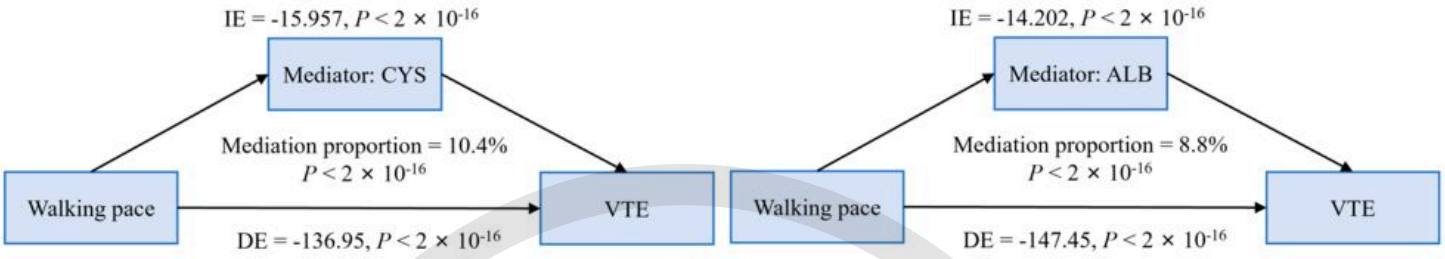
* The number of covariates adjusted are the same as that for Table 2.







Prospective cohort study



Mendelian randomization study