Joint association between endogenous sex hormones, genetic risk factors, and incident venous thromboembolism in women

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Committee:

Sara Lindstrom

Daniel Enquobahrie

Laura Harrington

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Abstract

Joint association between endogenous sex hormones, genetic risk factors, and incident venous thromboembolism in women

Samantha LeDuc

Chair of the Supervisory Committee:

Dr. Sara Lindstrom

Department of Epidemiology

Venous thromboembolism (VTE) is associated with genetic variations and the use of exogenous hormones such as oral contraceptives and hormone therapy. Previous studies have shown an inconsistent association between endogenous hormone concentrations and markers of coagulation and fibrinolysis, known risk factors of VTE. Further, little is known about the joint effects of genetic and endogenous risk factors. Using a nested case-control study design within the UK Biobank Cohort, we studied associations of endogenous hormone (estradiol, free testosterone, and SHBG) concentrations and genetic risk scores (GRS based on previously characterized VTE susceptibility variants in 37 genes) with risk of incident VTE among 661 premenopausal and 4,231 postmenopausal women (VTE cases n=126 and n=939 in pre- and postmenopausal women, respectively). We fit logistic regression models, examining the outcome of VTE by endogenous hormone concentrations, adjusting for age at assessment, BMI, diabetes diagnosis, cancer diagnosis, smoking status, and assessment site. We also evaluated the interaction of genetic risk scores and endogenous hormone concentrations with VTE incidence using and interaction term and log likelihood test, adjusting our models as previously specified. Higher endogenous SHBG concentrations were associated with a greater risk of VTE incidence, [(OR= 1.13 (95 % CI: 1.00, 1.43) per 30 nmol/L increment of SHBG; p = 0.27) in premenopausal women; OR= 1.13 (95 % CI: 1.00,

1.34) per 30 nmol/L increment of SHBG; p = 0.02) in postmenopausal women]. There was no evidence of associations of VTE incidence with free testosterone or estradiol concentrations. A Genetic Risk Score (GRS) based on 37 known VTE susceptibility variants was associated with increased VTE risk [OR= 2.03 (95% CI: 1.62, 2.60), p = 3.31 x 10^{-9} among premenopausal women; OR = 1.65 (95% CI: 1.51, 1.79), p< 2.0 x 10^{-16} among postmenopausal women].There did not appear to be a significant interaction between endogenous hormone concentrations and GRS with incident VTE risk [GRS and Testosterone interaction: OR = 0.954 (0.83, 1.11), p = 0.53 overall; GRS and SHBG interaction: OR = 1.001 (0.998, 1.004), p = 0.52 overall; GRS and detectible Estradiol: OR= 1.13 (0.88, 1.46), p = 0.34 overall]. Our findings of significant associations of SHBG concentration and GRS with VTE risk is consistent with existing literature.

Introduction

Venous thromboembolism (VTE), including pulmonary embolism (PE) and deep vein thrombosis (DVT), is a major cause of morbidity and mortality.[1] It is estimated that between 300,000 and 600,000 persons in the United States are diagnosed with VTE each year, with 10-30% dying within 30 days.[2] While VTE incidence is higher in men than women, the lifetime risk of VTE in women remains high, with an estimated age-adjusted lifetime incidence rate of VTE among women at 100 per 100,000.[1] Known risk factors for VTE include older age, greater body mass index (BMI), genetic predisposition, cancer diagnosis or treatment, surgery, bodily trauma, and hormone (exogenous or endogenous) related factors including pregnancy. [2–5]

It is well-established that use of exogenous hormones, including oral contraceptives (OC) and hormone therapy (HT), , i.e., OCs or HTs, is associated with a greater risk of VTE in pre- and postmenopausal women, respectively.[3,6] Furthermore, endogenous hormone concentrations have been associated with markers of coagulation and fibrinolysis[7,8] , and with incident risk of VTE (Table 1). [9–11] For instance, endogenous concentrations of sex hormone binding globulin (SHBG) have been linked to elevated concentrations of C-reactive protein (CRP), D-dimer, von Willebrand factor, and factor VIII[9,12], although reports of the associations have been inconsistent, with

some suggesting no evidence of an association.[13,14] Findings from studies on lifetime exposure to estrogen, estimated by the proxy measures of age at menopause and parity, suggest that higher exposure to endogenous estrogen is associated with greater risk of VTE. Compared to women with low parity (n <2) and normal menopausal timing (age = 46 – 54 years), women with low parity and early menopause (≤ 45 years) had the lowest risk of VTE (OR=0.60, 95% CI= 0.30–1.20)[15]. Notably, late menopause (≥55 years) and having given birth to more than two children was associated with the highest risk for VTE (OR= 3.41, 95% CI=1.46–9.25).[15] Few studies have evaluated endogenous sex hormones in relation to VTE risk (Table 1). One study found associations between endogenous concentrations of serum estradiol and VTE risk in postmenopausal women not using HT.[14] However, there is inconsistent evidence of the joint effect of genetic variation and endogenous hormone concentrations on VTE risk.[14]

Previous studies have found that variations in germline genetics are important contributors to an individual's risk of developing VTE.[16] Genetic variations in genes associated with coagulation factors, non-O blood type, and fibrinolytic pathways have been associated with VTE and can have a relatively large effect on overall risk.[17,18] Further, multiple susceptibility loci for VTE have been identified through genome-wide association studies (GWAS)[19]. VTE risk seems to be greatest for individuals having multiple genetic and lifestyle-based risk factors. For example, an early study found a significant interaction between exogenous hormones and the Factor V Leiden mutation with VTE risk, with a 30-fold increase in VTE risk in OC users who were Factor V Leiden mutation carriers when compared to non-OC-using non-carriers.[20]

Using data from the UK Biobank, we assessed the cross-sectional associations of circulating concentrations of free testosterone, estradiol, and sex hormone-binding globulin (SHBG) with incident VTE risk, by menopausal status, among women aged 40-69 at baseline who were not current users of OCs or HTs. Additionally, using previously identified single nucleotide polymorphisms (SNPs) associated with risk of VTE[19], we constructed a Genetic Risk Score (GRS) and assessed interactions between the GRS and the three hormone biomarkers (estradiol, free testosterone, and SHBG) on VTE risk, by menopausal status.

Methods

Study Design and Study Setting

This was a nested case-control study examining the association between circulating concentrations of endogenous hormones, genetic variations and incidence of VTE in pre- and postmenopausal women of European ancestry enrolled in the UK Biobank prospective cohort study. From the years 2006-2010, UK Biobank recruited 500,000 people between 40-69 years of age in the UK. Participants have undergone measures, provided blood, urine, and saliva samples for future analysis, provided detailed information and agreed to have their health followed.[21] Participants included in genetic variation analyses had GWAS data available.[22] Procedures for the current study were approved by UK Biobank under Project number 25298 (PI: Dr. Christopher Kabrhel).

Study Population

The current study was restricted to women participants in the UK Biobank study, ages 40-69 years at enrollment and currently using OCs or HTs. Study participants were limited to women of European ancestry as identified through ancestry markers. For the purposes of our analysis, we restricted the participants to pre- and postmenopausal women only, excluding perimenopausal women. Menopausal status was determined using responses from "Have you had your menopause (periods stopped)?", date of last menses, and age. Women were considered premenopausal if they did not report menopause and were under the age of 55. Included postmenopausal women confirmed menopause and reported having not had menses in the last two years. Women with less than two years since last menses were defined as perimenopausal and excluded from the current study. Women with missing data on menopausal status were classified as postmenopausal if they were over the age of 55 at time of collection (n=489); women ≤55 years of age with menopausal status missing were excluded (n=144). We further excluded women who underwent hysterectomy (n=582), current OC users (n= 56), current HRT users (n=392), and women currently pregnant (n=4) at time of enrollment/blood draw. The outcome of interest was VTE, which can further be categorized as DVT or PE. Cases were identified through ICD-10 codes (PE: I260, I269. DVT: I801, I80.2, I803, I822). After exclusions, the analytic population of premenopausal women (n=661) included 126 incident VTE cases and the analytic population of postmenopausal women (n=4,231) included 939 incident VTE cases. Since participant data have been deidentified, this study was exempt from IRB approval.

Data Collection

Blood and urine were collected from all participants; saliva was collected for a smaller subset. Data on biochemistry markers was determined from samples collected at recruitment and the first visit. All participants in UK Biobank have had genome-wide array genotyping performed. Additionally, participants have provided linkage to electronic health records, including death, hospital admissions, and primary care records.

Biomarkers

The exposures of interest were circulating concentrations of estradiol, free testosterone, and SHBG. For the purposes of this study, we used circulating hormone concentrations at time of blood collection and study enrollment in 2006-2010. Analyses involving testosterone and SHBG were conducted using continuous concentrations, among quartiles as defined by the distribution of values in controls, and in incremental concentrations (0.5 nmol/L in free testosterone; 30 nmol/L in SHBG).[23] Estradiol concentrations were analyzed using a dichotomous detectible/not detectible variable and continuously among detectible concentrations. Missing values of estradiol were differentiated from undetectable estradiol based on an individual's measurement for SHBG. If a participant had a measurement of 0 for estradiol and no measurement for SHBG, data were considered missing; if a participant had a measurement of 0 for estradiol and a measurement for SHBG present, the value for estradiol was considered 0 (undetectable).

Genetic variation

Blood for genotyping was collected from study participants at time of enrollment in 2006-2010. UK Biobank has generated GWAS data for all participants. The initial 50,000 individuals were genotyped with the Affymetrix UK BiLEVE array and the remaining 450,000 participants were genotyped with the Affymetrix UK Biobank Axiom Array, with the two different genotyping arrays having more than 95% overlap. Using 37 SNPs identified to be associated with VTE (Table 2), we calculated an aggregate GRS calculated using SNPs, consistent with Lindstrom et al[19] . For each individual, the GRS was calculated by summing up the product of the SNP-specific log odds ratio and the number of risk alleles carried for each SNP. We obtained SNP-specific log odds ratio estimates from a large VTE meta-analysis conducted for the included variants excluding this set of UK Biobank cases and controls. For each individual, the GRS was calculated using the product of select variants present, the number of risk alleles carried for each variant, and the log odds ratio for each variant. The GRS was standardized

to have a mean of 0 and a variance of 1 in our specific study population. For analyses involving GRS tertiles, GRS was split into low, medium, and high risk groups based on the distribution of GRS in controls and applied to the entire cohort.

Statistical analysis

Descriptive statistics were used to determine the distribution of selected participant characteristics. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between circulating concentrations of estradiol, free testosterone, and SHBG (coded either as continuous, or by control-based quartiles) and VTE (yes/no). Interactions between the individual continuous variables for circulating hormone concentrations and the GRS were assessed by including a product term in the model and conducting likelihood ratio tests. All analyses were adjusted for age at enrollment (continuous), body mass index at enrollment (continuous), diabetes (yes/no), self-reported cancer diagnosis (yes/no), and smoking status (ever, never). We conducted analyses within strata defined by menopausal status (premenopausal and postmenopausal), as well as the entire dataset adjusting for menopausal status. We considered a p-value <0.05 as statistically significant. All analyses were conducted using R version 3.6.1.

Results

Selected characteristics of our final study sample consisting of 1,065 VTE case and 3,827 control women of European ancestry aged 40-69 at time of assessment is shown in Table 3. Most women (N=4,231; 87%) were postmenopausal. Oveall, compared to controls, VTE cases had higher BMI (mean BMI 29.8 kg/m^2 among cases, 27.1 kg/m^2 among controls), and were more likely to be current smokers (10.2% in cases, 7.6% in controls), self-report diabetes diagnosis (6.9% in cases, 3.8% in controls), and self-report cancer diagnosis (15.3% in cases, 9.4% in controls). Similar differences were also observed when stratified by menopausal status.

In both premenopausal and postmenopausal women, mean concentrations of testosterone were slightly higher in cases than in controls (premenopausal: 1.22 nmol/L in cases, 1.16 nmol/L in controls; postmenopausal: 1.11 nmol/L in cases, 1.07 nmol/L in controls). SHBG concentrations were lower in cases than in controls,

regardless of menopausal status (premenopausal: 59.3 nmol/L in cases, 65.2 nmol/L in controls; postmenopausal: 56.7.2 nmol/L in cases, 59.3 nmol/L in controls). About 65% of premenopausal cases (and 67.4% controls) had detectible estradiol concentrations, whereas 3.8% of postmenopausal cases (and 2.47% controls) had detectible estradiol.

We observed a significant positive association between SHBG and risk of VTE among postmenopausal women (OR per 30 nmol/L increment=1.13; 95% CI: 1.00, 1.34; p= 0.02) (Table 4). A similar risk estimate was observed in premenopausal women, but the association was not statistically significant (OR=1.13; 95% CI: 0.89, 1.43; p= 0.27). Additionally, increasing quartiles of SHBG were positively associated with risk of VTE in postmenopausal women with the strongest association observed in Q4 [Q4: OR = 1.34 (1.04, 1.72); p-value for quartiles modeled continuously=0.03]. Circulating concentrations of testosterone were not associated with incident VTE among either premenopausal [OR per 0.5 nmol/L increment=1.07 (95% CI: 0.87, 1.29), p= 0.51] or postmenopausal [OR= 1.01 (95% CI: 0.93, 1.08) p=0.89] women, though estimates suggested positive association, they were not statistically significant. No significant association was observed between detectible concentrations of estradiol and risk of VTE among either premenopausal or postmenopausal women [OR = 0.95 (95% CI: (0.59, 1.55), p = 0.83 in premenopausal women; OR = 1.15 (95% CI: 0.72, 1.81), p = 0.54 in postmenopausal women].

We observed a strong significant association between the GRS[19] and VTE in both premenopausal and postmenopausal women [OR=2.03 (95% CI: 1.62, 2.60), p= 3.31 $x10^{-9}$ in premenopausal women; OR=1.65 (95% CI: 1.51, 1.79) per 1 SD increase, p= < 2.0 x 10^{-16} in postmenopausal women)] (Table 5). While there was no statistically significant interaction between GRS and endogenous hormones on risk of VTE, it was observed that GRS and SHBG were weakly associated with increased risk of VTE [p =0.46], testosterone was slightly protective [p =0.48], and detectible estradiol was non-significantly associated with increased risk [p = 0.27] in premenopausal women.

Discussion

In this study of circulating concentrations of endogenous hormones and incident VTE risk in women, we observed a significant association between SHBG concentrations and VTE risk in postmenopausal women [OR=1.13 (95% CI: 1.00, 1.34), p=0.02 per 30 nmol/L SHBG]. This same risk estimate was observed in premenopausal women [OR=1.13 (95% CI: 0.89, 1.43), p= 0.27], but was not significant, potentially due to smaller sample size. Overall, it appears that increasing concentrations of SHBG are positively associated with risk of VTE in postmenopausal women, with the highest risk observed when comparing Q4 to Q1 SHBG concentrations [Q4 vs Q1: 1.34 (95% CI:1.04, 1.72); p-value for quartiles modeled continuously=0.03].

In contrast to SHBG, we did not observe any overall or menopausal status stratified significant associations between circulating concentrations of testosterone or estradiol and risk of VTE. In premenopausal women, we observed a suggestive positive association between increasing testosterone quartiles and VTE risk and no association among postmenopausal women. In premenopausal women, the greatest risk of VTE was observed in the second and fourth testosterone quartiles [Q2 vs Q1: OR=2.15 (1.03, 4.68); Q3 vs. Q1: OR=1.57 (0.77, 3.37); Q4 vs. Q1: OR=1.63 (0.82, 3.43]. Although we observed a significant association in the second quartile, we acknowledge that this may be a chance finding, or that our limited sample size of premenopausal women may result in false negatives in the other quartiles.

The observed null association between endogenous testosterone, estradiol, and VTE risk, as well as a slightly higher risk of VTE with increasing SHBG concentrations are consistent with reports from most, but not all, previous studies.[9,10,14] In a population-based case-control study among women under the age of 45, Scheres et al.[10] found no evidence of a significant association between total testosterone and VTE risk, but observed an association between increasing estradiol concentrations and VTE risk with the largest risk in the fourth quartile as compared to the first quartile [OR=1.6 (95% CI: 1.0-2.5)]. It is worth noting that their study included more cases among premenopausal women (N=369) than the 126 cases among premenopausal women included in our dataset. Additionally, Scheres et al identified a positive association between SHBG concentrations and VTE, with the greatest risk among the fourth quartile [Q4 vs Q1: OR = 2.0 (1.2-3.2)]. This association is consistent with our findings, although we did not observe as strong of an association with risk.

In a prospective cohort study among postmenopausal women not using HT (N= 3,051) by Roetker et al.,[14] a similar trend was observed, with the fourth quartile of SHBG showing a non-significant higher risk of VTE [Q4 vs Q1: HR=1.4 (95% CI: 0.9, 2.20)]. This study also confirmed the established inverse relationship between BMI and SHBG [average BMI by SHBG quartiles: ($kg/m^{2)}$, by SHBG quartile; Q1: 33 kg/m^2, Q2: 31 kg/m^2, Q3: 29 kg/m^2, Q4: 26 kg/m^2) among female participants not using HT. Roetker et al. performed analyses of the association between SHBG and VTE risk with and without adjusting for BMI and observed a change in the strength of association in the fourth quartile when not adjusting for BMI [HR = 1.1(0.7, 1.6)] and with BMI adjustment [HR = 1.4 (0.9, 2.2]. In our study, SHBG goes from being positively associated with VTE risk when adjusted for BMI [OR= 1.00 (0.80, 1.26) per 30 nmol/L in premenopausal women; OR = 1.09 (1.01, 1.19) per 30 nmol/L in postmenopausal women] to being a protective factor against VTE when BMI is not included in the model [OR = 0.73 (0.60, 0.88) per 30 nmol/L in premenopausal women; OR = 0.93 (0.86, 1.00) per 30 nmol/L in postmenopausal women]. This is clear evidence of confounding, supporting the fact that VTE risk is correlated with increased BMI and obesity is associated with lower concentrations of SHBG, both of which are supported by the literature. [14,24]

Our findings of a greater risk of VTE with increasing GRS in premenopausal women [Medium vs Low: OR = 1.89 (0.92, 3.93); High vs Low: OR = 3.83 (2.13, 7.20)] and postmenopausal women [Medium vs Low: OR = 1.48 (1.17, 1.86); High vs Low: OR = 2.95 (2.39, 3.66)] are consistent with previous findings by Crous-Bou et al. [Medium vs Low: OR = 1.33 (1.12–1.57); High vs Low: OR = 2.02 (1.73 – 2.34)].[5] We would expect to observe a stronger association between GRS and VTE in younger women as these women have less influence from non-genetic risk factors, and our results are consistent with this. Postmenopausal women overwhelmingly had a higher proportion of cancer diagnosis and diabetes diagnosis than their premenopausal cohort members, which are known risk factors for VTE. Additionally, postmenopausal cases have a lower mean BMI than premenopausal women; the inverse association between SHBG and BMI may have attenuated the observed association with VTE risk. No significant interaction was observed between increased GRS and endogenous hormones with risk of VTE; joint GRS and SHBG were non-significantly associated with increased risk of VTE [OR = 1.003 (0.994, 1.013), p =0.46], joint GRS and testosterone were non-significantly associated [OR = 0.87 (0.59, 1.33), p =0.48], and detectible

estradiol was non-significantly associated with higher risk [OR = 1.33 (0.80, 2.21), p = 0.27] in premenopausal women. Again, our limited sample size may have left us underpowered to detect a significant interaction.

Some limitations of our study deserve mention. UK Biobank participants must be aged 40-69 at the time of enrollment. Women typically experience menopause in their late 40s or early 50s, so our sample size of pre-menopausal women was limited. Many postmenopausal estradiol levels were below the lower limit of detection, further decreasing our study power in the postmenopausal estradiol analyses. Our study population was comprised entirely of women of European ancestry, limiting generalizability of findings. Data on menopausal status, smoking status, OC use, HT use, and BMI were collected at time of enrollment. All of these may change after enrollment and lead of misclassification of important covariates.

While it is established that specific genetic risk factors and use of exogenous hormones are associated with VTE risk, there is little understanding of endogenous hormones on VTE risk in women. Our findings suggest potential associations of SHBG concentration with VTE risk among women. Further investigation is warranted regarding the interaction between inherent genetic risk, endogenous hormones, and VTE risk, both among women and men. As increased SHBG appears to be associated with increased VTE risk in premenopausal and postmenopausal women, more evidence should be collected on the association between BMI, SHBG, and VTE risk across menopausal status. These findings may be useful as a screening tool to understand an individual's inherent risk of VTE and may inform decisions surrounding use of OCs or HT.

Tables

Table 1: Previous studies examining endogenous hormones and VTE in women

First Author	Title	Sample Size	Population	Hormones	Results	Study Design
Scheres LJJ, 2019	Endogenous sex hormones and risk of venous thromboembolism in young women	665 women (368 cases; 269 controls)	Women aged ≤45 years from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) case-control study	Sex hormone–binding globulin (SHBG), estradiol, follicle-stimulating hormone (FSH) and testosterone	no apparent association between total testosterone and FSH and VTE risk. Somewhat increased risk of VTE in the fourth quartile of estradiol (OR 1.6; 1.0-2.5). Increase risk of VTE with increasing quartiles of SHBG; OR 2.0 (1.2-3.3) comparing the fourth quartile to the first.	Population based case-control
Holmegard, H.N., 2014	Endogenous sex hormones and risk of venous thromboembolism in women and men	4658 women and 4673 men	Men and women aged 20- 80+ years enrolled in 1981–1983 Copenhagen City Heart Study. Participants were of European ancestry (Danish descent).	total estradiol and total testosterone	Adjusted hazard ratios of VTE for individuals with estradiol levels >75th vs. ≤25th percentile were 0.84 (95%CI, 0.25–2.85) in premenopausal women and 1.05 (0.53–2.08)in postmenopausal women. For testosterone, risk estimates for pre- and post-menopausal women were 0.64 (0.03–12.32) and 1.11 (0.66–1.86), respectively	Prospective observational cohort
Roetker, N., 2018	Prospective Study of Endogenous Hormones and Incidence of Venous Thromboembolism: The Atherosclerosis Risk in Communities Study	3,051 non-HRT-using women, 1,414 HRT-using post-menopausal women and 3,925 men	Participants in ARIC in four US communities (Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi; and Minneapolis, Minnesota). Multiethnic study.	dehydroepiandrosterone sulphate (DHEAS), testosterone and sex hormone-binding globulin (SHBG)	No support for significant association between testosterone, DHEAS, or SHBG in post-menopausal women not using HRT.	Prospective, multi-center, population-based cohort
Simon, T., 2006	Indicators of lifetime endogenous estrogen exposure and risk of venous thromboembolism	608 postmenopausal women (191 cases and 417 controls). No information on ancestry of participants.	Women aged 45-70 enrolled in the ESTHER study.	Estrogen exposure, as determined by age at menopause [classified as early (< 45 years), normal (46–54 years) and late menopause (>55 years)] and parity	With women with normal menopause as reference, OR for VTE was 0.59 (95% CI ¼ 0.36–0.97) and 2.53 (95% CI ¼ 1.28–4.99) for women with early menopause and late menopause, respectively. OR for VTE was higher for	Case-control

					women with more than two children when compared with those with less than or equal to two children.	
Williams, M., 2016	Association of Serum Sex Hormones with Hemostatic Factors in Women On and Off Hormone Therapy: The Multiethnic Study of Atherosclerosis	1,933 women not using HT, 945 women using HT. Multiethnic study.	Multiethnic Study of Atherosclerosis (MESA) is a longitudinal cohort study	Total testosterone, bioavailable testosterone, estradiol, DHEA, SHBG	Did not directly assess VTE risk but examined the association between sex hormone levels and thrombotic biomarkers. HT nonusers had more prothrombotic associations between increased sex hormone levels and thrombotic biomarkers, compared to users.	Cross-sectional analysis of women in large multiethnic community-based cohort

Table 2: Genetic variants used to compute Genetic Risk Score (GRS) from Lindstrom et al., 2019 [17]

Genomic position	Variant	Locus	Effect allele	Other allele	Log odds ratio	SE	P
1:169519049	rs6025	F5 (exon)	t	c	0.87	0.03	1.43E-188
1:169511755	rs4524	F5 (exon)	t	c	0.13	0.01	5.29E-29
1:207282149	rs2842700	C4BPA (intron)	a	c	0.14	0.02	9.49E-11
1:230986407	rs145470028	C1orf198 (intron)	t	g	-0.25	0.04	3.04E-08
2:68619981	rs1867312	PLEK (intron)	a	c	-0.06	0.01	8.79E-10
4:155525695	rs2066864	FGG (intron)	a	g	0.18	0.01	1.22E-57
4:187207381	rs2289252	F11 (intron)	t	c	0.17	0.01	1.05E-63
4:187192481	rs2036914	F11 (intron)	t	c	-0.16	0.01	1.26E-53
4:187204937	rs4253421	F11 (intron)	a	g	-0.22	0.02	1.56E-40
5:38707871	rs4869589	OSMR-AS1 (intron)	t	g	0.08	0.01	6.56E-11
6:147701133	rs9373523	STXBP5 (intron)	t	g	-0.06	0.01	2.17E-09
8:27898452	rs12675621	NUGGC (intron), SCARA5 (upstream)*	a	g	-0.06	0.01	8.29E-09
8:106590705	rs4541868	ZFPM2 (intron)	a	c	-0.09	0.01	1.80E-13
9:136131188	rs8176749	ABO (exon)	t	c	0.20	0.02	1.70E-26
9:136137106	rs687289	ABO (intron)	a	g	0.29	0.01	1.73E-172
9:136141870	rs2519093	ABO (intron)	t	c	0.34	0.01	1.89E-168
9:136154168	rs579459	ABO (intron)	t	c	-0.31	0.01	8.61E-147
10:71245276	rs78707713	TSPAN15 (intron)	t	c	0.18	0.02	2.62E-30
10:121010256	rs10886430	GRK5 (intron)	a	g	-0.11	0.02	1.23E-12
11:46933311	rs191945075	LRP4 (intron), F2 (downstream)	a	g	0.62	0.05	9.55E-32
11:46760756	rs3136516	F2 (intron)	a	g	-0.08	0.01	2.02E-15
12:6153738	rs1558519	VWF (intron)	a	g	-0.08	0.01	1.79E-13
12:6128443	rs216311	VWF (exon)	t	c	-0.03	0.01	1.92E-02
12:123667354	rs2851436	MPHOSPH9 (intronic)	t	g	0.06	0.01	2.12E-07
14:58844526	rs11158204	Intragenic	t	c	-0.07	0.01	2.49E-08
16:81870969	rs12445050	PLCG2 (intron)	t	c	0.10	0.01	4.44E-13
17:1966457	rs1048483	SMG6 (intron)	t	c	0.06	0.01	1.08E-09
17:7207887	rs12450494	Intergenic, EIF5A (upstream)	a	t	0.06	0.01	2.47E-08
19:10740871	rs4548995	SCL44A2 (intron)	c	g	0.11	0.01	8.96E-19
19:13258290	rs7508633	STX10 (intron)	a	g	-0.06	0.01	1.97E-09
19:55511873	rs1671135	NLRP2 (intron), GP6 (downstream)	c	g	0.08	0.01	2.35E-09
20:33764554	rs867186	PROCR (exon)	a	g	-0.10	0.02	1.44E-09
20:33745676	rs6088735	PROCR (intron)	t	c	0.08	0.01	2.30E-10
14:92309229	rs1884841	TCN2	a	g	0.04	0.01	1.18E-05
3:186459927	rs710446	KNG1	t	c	-0.05	0.01	5.74E-07
23:138633280	rs6048	F9	a	g	0.06	0.01	7.83E-08
23:154424170	rs143478537	F8	c	g	-0.08	0.02	9.17E-08

Table 3: Selected characteristics of study participants, overall and by menopausal status

	Premenopausal women (n=661)						Postmenopausal women (n=4231)						Overall (n=4892)					
	VTE case (n=126)			Control (n=535)			VTE case (n=939)			Control (n=3292)			VTE case (n=1065)			Control (n=3827)		
	Mean	SD	IQR	Mean	SD	IQR	Mean	SD	IQR	Mean	SD	IQR	Mean	SD	IQR	Mean	SD	IQR
Age at recruitment (years)	48.3	5.46	--	47.8	4.83	--	62.5	4.6	--	62.3	4.43	--	60.8	6.57	--	60.3	6.73	--
BMI	30.6	7.58	--	26.8	5.39	--	29.6	5.8	--	27.2	5.08	--	29.8	6.04	--	27.1	5.13	--
Current smoker, %	11.60%	--	--	6.93%	--	--	10.45%	--	--	6.93%	--	--	10.15%	--	--	7.58%	--	--
Diabetes diagnosis, %	4.76%	--	--	2.80%	--	--	6.64%	--	--	3.84%	--	--	6.85%	--	--	3.83%	--	--
Cancer Diagnosis, %	7.14%	--	--	3.18%	--	--	16.36%	--	--	10.63%	--	--	15.27%	--	--	9.35%	--	--
Testosterone																		
Testosterone (continuous)	1.22	0.48	0.572	1.16	0.563	0.696	1.11	0.574	0.604	1.07	0.569	0.614	1.12	0.563	0.613	1.08	0.569	0.634
SHBG																		
SHBG (continuous)	59.3	34.9	43.2	65.2	27.9	36.2	56.7	27.7	35.3	59.3	27.5	34.4	57	28.7	36.3	60.1	27.6	34.5
Estradiol																		
Estradiol (continuous)	377	473	495	356	433	470	12.9	98.5	0	7.96	61.8	0	59.2	227	0	58.3	213	0
Estradiol (detectible)	65.00%	--	--	67.36%	--	--	3.76%	--	--	2.47%	--	--	11.55%	--	--	11.87%	--	--

Table 4. Risk of incident VTE by endogenous hormone levels among premenopausal and postmenopausal women in the UK Biobank

	Premenopausal women (n=661; cases: n=126; controls: n= 535)		Postmenopausal women (n=4231; cases: n=939; controls: n=3292)		Overall (n=4892) [2]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Testosterone[1]						
Testosterone (continuous)	1.14 (0.76, 1.68)	0.51	1.01 (0.87, 1.17)	0.89	1.02 (0.90, 1.17)	0.74
Testosterone (quartiles overall)	1.07 (0.88, 1.31)	0.51	1.02 (0.95, 1.10)	0.59	1.03 (0.96, 1.11)	0.44
Testosterone Q1 (ref)	*ref*	--	*ref*	--	*ref*	--
Testosterone Q2	2.15 (1.03, 4.68)	0.05	0.95 (0.74, 1.22)	0.67	1.05 (0.83, 1.33)	0.69
Testosterone Q3	1.57 (0.77, 3.37)	0.23	1.21 (0.96, 1.54)	0.11	1.24 (0.99, 1.56)	0.06
Testosterone Q4	1.63 (0.82, 3.43	0.18	0.99 (0.77, 1.26)	0.92	1.04 (0.83, 1.32)	0.71
Testosterone (per 0.5 nmol/L increment)	1.07 (0.87, 1.29)	0.51	1.01 (0.93, 1.08)	0.89	1.02 (0.95, 1.08)	0.74
SHBG[1]						
SHBG (continuous)	1.004 (0.996, 1.012)	0.27	1.004 (1.00, 1.01)	0.02	1.004 (1.00, 1.01)	0.01
SHBG (continuous, w/o adjusting for BMI) [3]	0.99 (0.98, 1.00)	0.05	0.997 (0.99, 1.00)	0.05	0.996 (0.994, 0.992)	0.01
SHBG (quartiles)	1.00 (0.80, 1.26)	0.98	1.09 (1.01, 1.19)	0.03	1.08 (1.00, 1.17)	0.04
SHBG (quartiles, w/o adjusting for BMI) [3]	0.73 (0.60, 0.88)	0.001	0.93 (0.86, 1.00)	0.04	0.90 (0.84, 0.96)	0.003
SHBG Q1 (ref)	*ref*	--	*ref*	--	*ref*	--
SHBG Q2	0.48 (0.24, 0.95)	0.04	1.04 (0.83, 1.30)	0.73	0.96 (0.78, 1.19)	0.73
SHBG Q3	0.65 (0.32, 1.30)	0.22	1.09 (0.86, 1.38)	0.49	1.02 (0.81, 1.27)	0.89
SHBG Q4	0.90 (0.46, 1.78)	0.77	1.34 (1.04, 1.72)	0.02	1.29 (1.02, 1.62)	0.03
SHBG (per 30 nmol/L increment)	1.13 (0.89, 1.43)	0.27	1.13 (1.00, 1.34)	0.02	1.13 (1.00, 1.34)	0.01
Estradiol[1]						
Estradiol (continuous) [4]	1.00 (0.999, 1.001)	0.34	1.00 (1.00,1.003)	0.27	1.00 (0.999, 1.001)	0.24
Estradiol (detectible)	0.95 (0.59, 1.55)	0.83	1.15 (0.72, 1.81)	0.54	1.15 (0.86, 1.53)	0.33

1: Adjusted for: BMI (continuous), age, self-reported diabetes, self-reported cancer, and smoking status (never, current, previous).
2: Additionally adjusted for menopausal status
3: Adjusted for: age, self-reported diabetes, self-reported cancer, and smoking status (never, current, previous).
4: continuous among detectible levels only

Table 5. Genetic Risk Score on risk of incident VTE among premenopausal and postmenopausal women in the United Kingdom.

	Premenopausal women (n=512; cases: n =110, controls: n= 402)		**Postmenopausal women (n=3439; cases: n= 840, controls n=2599)**		**Overall (n=3951)[2]**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Genetic Risk Score [1]						
GRS (standardized, w/o hormones)	2.03 (1.62, 2.60)	3.31 e -9	1.65 (1.51, 1.79)	< 2.0 e -16	1.68 (1.55, 1.81)	2.00 e-16
Low GRS	*ref*	--	*ref*	--	*ref*	--
Medium GRS	1.89 (0.92, 3.93)	0.083	1.48 (1.17, 1.86)	9.0 e-4	1.51 (1.21, 1.88)	2.15 e -4
High GRS	3.83 (2.13, 7.20)	1.42 e -5	2.95 (2.39, 3.66)	< 2e-16	3.02 (2.48, 3.70)	< 2e-16
Joint Association: GRS and Hormone Concentrations						
Testosterone[1]						
Continuous	0.87 (0.59, 1.33)	0.48	0.96 (0.83, 1.13)	0.65	0.954 (0.83, 1.11)	0.53
SHBG[1]						
Continuous	1.003 (0.994, 1.013)	0.46	1.00 (0.997, 1.00)	0.83	1.001 (0.998, 1.004)	0.52
Estradiol[1]						
Continuous[3]	1.001 (1.000, 1.002)	0.03	1.007 (1.001, 1.019)	0.087	1.001 (1.000, 1.002)	0.01
Detectible	1.33 (0.80, 2.21)	0.27	0.90 (0.57, 1.44)	0.66	1.13 (0.88, 1.46)	0.34

1: Adjusted for: BMI (continuous), age, self-reported diabetes, self-reported cancer, and smoking status (never, current, previous).
2: Additionally adjusted for menopausal status
3: continuous among detectible levels only

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