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Clinical Research Article

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Association of Genetically Predicted Serum Estradiol With Risk of Thromboembolism in Men: A Mendelian Randomization Study

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Abbreviations: CYP19A1, aromatase; GWAS, genome-wide association study; IVW, inverse variance weighting; MR, mendelian randomization; SNV, single-nucleotide variation.

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Abstract

Context: An association was recently reported between genetic markers related to high testosterone and increased risk of thromboembolism in men, but a possible causal role of estradiol for risk of thromboembolism in men remains unknown.

Objective: This work aimed to determine whether endogenous estradiol has a causal role in thromboembolism in men.

Methods: A 2-sample mendelian randomization study using gene-based genetic instruments assessed the association between endogenous estradiol genetically predicted by 22 variants in the aromatase *CYP19A1* gene region and the risk of thromboembolism (5815 cases) in 170 593 unrelated men of White ancestry in the UK Biobank. The main outcome measure included thromboembolism based on self-reports, hospital episodes, and death.

Results: Endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region was inversely associated with the risk of thromboembolism (odds ratio per SD increase in estradiol 0.74; 95% CI, 0.62-0.90). In contrast, genetic variants in the *JMJD1C* gene, used as a predictor of high endogenous testosterone, were associated with an increased risk of thromboembolism (odds ratio per SD increase in testosterone 1.39; 95% CI, 1.12-1.72). Subsequent explorative analyses evaluating potential repercussions of

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thromboembolism revealed that endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region was inversely associated with the risk of ischemic stroke (0.68; 95% CI, 0.49-0.95) but not myocardial infarction (0.97; 95% CI, 0.84-1.13).
Conclusion: Genetically predicted estradiol was inversely associated with the risk of thromboembolism and ischemic stroke in men. The ratio between testosterone and estradiol, determined by CYP19A1 activity, may contribute to the overall impact of sex steroids on thromboembolism in men.

Key Words: testosterone, estradiol, thromboembolism, mendelian randomization, aromatase

The number of off-label testosterone prescriptions has increased considerably worldwide, often in middle-aged and older men in whom low testosterone levels have not been demonstrated and despite clinical trials having shown only modest benefit (1). In addition, evidence on the long-term safety of testosterone treatment is lacking and to date, no clinical trial has been adequately powered or designed to assess adverse cardiovascular events (2). Several epidemiological studies have suggested that endogenous sex hormones, and in particular the predominant male sex hormone testosterone, are implicated in cardiovascular events in men, with low levels being associated with increased disease risk (3-7). Nevertheless, these observational cohort studies are difficult to interpret because of the heterogeneity in the studies and their susceptibility to residual confounding and reverse causality (8).

One way to overcome confounding and establish causality is by mendelian randomization (MR). This method uses genetic variants that have a specific influence on possible risk factors to assess associations with disease outcomes. MR avoids some of the limitations of observational studies (because genetic information is free from confounding) and is not affected by disease status, thereby avoiding reverse causation bias (9).

Sex steroids may influence the risk of cardiovascular disease via an effect on thromboembolism. Using MR, an association was recently reported between genetic markers related to high testosterone and increased risk of thromboembolism in men (10). As testosterone is directly metabolized to estradiol by the aromatase (CYP19A1) enzyme, we hypothesized that endogenous estradiol also might influence the risk of thromboembolism in men. Even though estradiol has long been considered the "female hormone," levels of serum estradiol in men are higher than those in postmenopausal women, and not only testosterone but also estradiol is proposed to be crucial for men's health (11). Using MR studies, we have recently shown that genetically predicted serum estradiol, but not testosterone, is directly causally associated with bone mineral density (12) and inversely causally associated with fracture risk (13) in men. These studies demonstrate that the causal role of endogenous testosterone and estradiol may differ for certain phenotypes. In addition, they highlight the importance of endogenous estradiol in men, supporting previous findings in men with aromatase or estrogen receptor-α deficiency (14). The genetic determinants explaining the majority of the identified variance in circulating estradiol levels are located in the *CYP19A1* gene region (12). Importantly, the alleles of the genetic variants in the *CYP19A1* gene region that are associated with increased serum estradiol are not associated with increased serum testosterone, making it possible to use these in a gene-based MR to separate the causal effects of estradiol from those of testosterone (12, 15, 16). Previous prospective, population-based observational studies have not found an association between endogenous serum estradiol and the risk of thromboembolism in men (17, 18). To evaluate the potential causal association between endogenous estradiol and the risk of thromboembolism in men, we conducted an MR study in the UK Biobank using genetic variants in the *CYP19A1* gene region as instrumental variables. The use of oral contraceptives or hormone replacement therapy is associated with an increased risk of venous thromboembolism in women (19, 20), while the risk associated with transdermal formulations is less (21). We therefore hypothesized that endogenous estradiol would be directly associated with risk of thromboembolism in men.

Materials and Methods

We conducted a 2-sample MR study to determine the possible causal associations between endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region and the risk of the primary outcome thromboembolism in men in the UK Biobank. In the same cohort and using the same gene-based MR methodology, we examined the association between genetic variants in the *JMJD1C* gene, used as a predictor of endogenous testosterone, and thromboembolism. Finally, we investigated the associations of endogenous estradiol with potential repercussions of thromboembolism, namely ischemic stroke and myocardial infarction. We obtained genetic predictors

of serum estradiol in the *CYP19A1* gene region from the largest available genome-wide association study (GWAS) (Supplementary Fig. 1 and Supplementary Table 1) (12, 22) and also used variants in the *JMJD1C* gene, associated with endogenous testosterone (Supplementary Table 2) (10, 22, 23). We used the UK Biobank to assess the associations of estradiol with the primary outcome thromboembolism and the subsequent exploratory outcomes venous thromboembolism, arterial embolism and thrombosis, ischemic stroke, and myocardial infarction (24). We used a gene-based MR method to estimate the causal association for endogenous estradiol, predicted by multiple genetic variants in the biologically plausible *CYP19A1* gene region, with thromboembolism. The inclusion of multiple variants in partial linkage disequilibrium can explain a greater proportion of variance in the exposure, leading to a more powerful MR analysis (25).

Participants

Genetic predictors of endogenous estradiol in men

Genetic predictors of estradiol were obtained from a GWAS meta-analysis of serum estradiol comprising 11 097 male participants of European origin from 9 epidemiological cohorts (12). Exclusion criteria included chemical or surgical castration and/or medications affecting sex hormones such as steroid 5-α reductase inhibitors and sex hormone antagonists. Measurements of estradiol were mainly performed by gold-standard mass spectrometry (gas chromatography–mass spectrometry or liquid chromatography–tandem mass spectrometry); this is a strength because immunoassay-based techniques have a known questionable specificity, especially in the lower concentration range (26-29). Genotypes from all participating cohorts were imputed to HapMap 3 CEU. The *CYP19A1* gene region, coding for the biologically plausible enzyme aromatase, displayed by far the most robust genome-wide significant association signals with serum estradiol. In total 228 available genetic variants from the *CYP19A1* gene region (within 100 kb from the top GWAS signal rs727479) (http://www.gefos.org/?q=content/estrogen-gwas-2018) (12) were checked for validity as instrumental variables using individual level data from the UK Biobank, according to the following exclusion criteria: GWAS *P* value greater than .05, imputation information score less than 0.6; departure from the Hardy-Weinberg equilibrium at Bonferroni-corrected significance; or violation of the MR assumption that the genetic variant should be unrelated to factors potentially confounding any association with the outcomes, including baseline age, body mass index, socioeconomic status (Townsend index and educational level), and lifestyle factors (smoking and drinking) at Bonferroni-corrected significance. Single-nucleotide variation (SNV; formerly single-nucleotide polymorphism [SNP]) effects were harmonized to have the same effect allele on the same strand in both samples. We excluded 3 palindromic SNVs with allele frequencies close to 0.5 (see Supplementary Fig. 1) (22) because we could not determine the effect direction of these SNVs with certainty. Of the remaining genetic variants, we selected those with the lowest *P* value having a pairwise squared correlation (r^2) less than 0.4, resulting in 22 validated genetic variants predicting serum estradiol to be used in the MR (see Supplementary Fig. 1 and Supplementary Table 1) (22). We aligned the effect allele of each genetic variant on the serum estradiol increasing allele. Population-specific correlations between variants were estimated with LDlink (30) using the 1000 Genomes Project (phase 3) and the British in England and Scotland population. As described earlier, the selected SNVs had a pairwise r^2 less than 0.4 (see Supplementary Fig. 1) (22) and they therefore contributed with both common and unique information on serum estradiol. In the gene-based MR analyses, we subsequently accounted for remaining correlations among selected genetic variants; thereby this methodology captures the total cumulative unique but not overlapping association information with high precision from multiple SNVs within the *CYP19A1* gene region (10, 16).

Genetic associations with the primary outcome thromboembolism and exploratory outcomes

From 2006 to 2010, the UK Biobank recruited around 500 000 individuals aged 37 to 73 years from across the United Kingdom. Participants provided biological samples, completed questionnaires, underwent assessments, and participated in nurse-led interviews. Follow-up using record linkage to all health service encounters and mortality data is ongoing (31, 32). Genotyping was undertaken with 2 very similar arrays from Affymetrix: the UK BiLEVE array and the UK Biobank Axiom array. Genotype imputation to a reference set combining the UK10K haplotype and the Haplotype Reference Consortium reference panels was performed (33, 34). In the present study, imputed genotypes version 2 were used. To reduce confounding by population stratification, we restricted our analysis to participants of White ancestry and excluded individuals for the following reasons: withdrawn consent; sex mismatch (derived by comparing genetic and reported sex); sex chromosome aneuploidy; poor quality genotyping (missing rate > 1.5%); or relatedness (unrelated samples used in the UK Biobank principal component analysis calculations were selected). Of 229 167 available men from the UK Biobank, 170 593 men of White ancestry remained after these sample and genetic controls. The UK Biobank has received ethical approval from the Northwest Multicentre Research Ethics

Committee, and informed consent was obtained from all participants. The present research was approved by the UK Biobank Research and Access Committee (application number 26947).

Exposures

The evaluated main exposure was genetically predicted serum estradiol (picogram per milliliter; pg/mL). To obtain a more interpretable metric, we report effect sizes by 1 SD increase in estradiol (1 SD = 9.6 pg/mL from the Framingham Heart Study) (12). In addition, we assessed log-transformed endogenous testosterone (nanomole per liter; nmol/L) as exposure, predicted by 9 genetic variants in the *JMJD1C* gene. Because the SD for log-transformed testosterone was not given in the published GWAS (23), we estimated the SD using the method described by Rietveld et al (35).

Outcomes

We evaluated the primary outcome thromboembolism and the exploratory outcomes (venous thromboembolism, arterial embolism and thrombosis, ischemic stroke, and myocardial infarction) in the UK Biobank based on self-reported events, primary hospital episodes, and primary cause of death as given in Supplementary Table 3 (22).

Statistical analysis

Causal effects

We used a gene-based MR method to estimate the association of endogenous estradiol predicted by multiple genetic variants in the biologically plausible *CYP19A1* gene region with the primary outcome thromboembolism and the exploratory outcomes. Associations of genetic variants with the outcomes were estimated in the UK Biobank using logistic regression adjusted for age, genotyping array, and the top 10 principal components. Causal effect estimates were obtained using fixed effects inverse variance weighting (IVW) that account for correlations among genetic variants within the evaluated gene region (25, 36). Because we are using correlated variants from single gene regions, we first used the Q statistic (37) to identify the presence of potential pleiotropy. As an additional tool to identify potential presence of directional pleiotropy, we used Egger regression accounting for correlated genetic variants (36). All MR analyses were performed using the MendelianRandomization package in R, version 3.4.2.

Power calculation

For the primary analysis, the association between endogenous estradiol predicted by variants in the *CYP19A1* gene region and risk of thromboembolism, the study had 80% power to detect an overall odds ratio of greater than 1.22 (or < 0.79 if inverse association) per SD increase in serum estradiol (see Supplementary Fig. 2) (22). The power calculation assumes that the 22 used genetic variants in the *CYP19A1* gene region collectively explain 3.0% of the variance in serum estradiol as estimated in the Gothenburg part of MrOS (Osteoporotic Fractures in Men) Sweden with serum estradiol analyzed by gas chromatography–mass spectrometry (864 older men). The related F statistic for the instrumental variable is 251.2. Power calculations were performed using an online tool (https://shiny.cnsgenomics.com/mRnd/) applying a noncentrality parameter approach adapted for binary outcomes using an approximate linear model on the observed binary scale.

Results

This study aimed to determine possible causal associations between endogenous estradiol and the risk of thromboembolism in men. We first validated genetic instruments for endogenous estradiol in the *CYP19A1* gene region derived from a recent estradiol GWAS meta-analysis on serum estradiol measured mainly by state-of-the-art mass spectrometry methodology (http://www.gefos.org/?q=content/estrogen-gwas-2018) (12). Twenty-two variants fulfilled the criteria of being associated with the exposure ($P < .05$), high imputation quality, Hardy-Weinberg equilibrium, not being associated with potential confounders, and not being highly correlated (see Supplementary Fig. 1 and Supplementary Table 1) (22). In the gene-based MR analyses, we subsequently accounted for remaining correlations among selected genetic variants. We also examined the association with thromboembolism using 9 genetic variants in the *JMJD1C* gene, used as a predictor of endogenous testosterone and selected using a similar strategy as previously described (see Supplementary Table 2) (10, 22, 23).

Of 229 167 available men from the UK Biobank, 170 593 men of White ancestry remained after sample and genetic controls, and these had a mean age of 57.0 years (Table 1). Of these 170 593 men, 5815 had the primary outcome thromboembolism and the study had 80% power to detect an overall odds ratio of greater than 1.18 (or < 0.83 if inverse association) per SD increase in serum estradiol for thromboembolism in the UK Biobank (see Table 1 and Supplementary Fig. 2) (22).

Endogenous Estradiol Was Inversely Causally Associated With Risk of Thromboembolism

Using fixed-effects IVW MR adjusted for SNV correlations, we observed that endogenous estradiol genetically

predicted by variants in the *CYP19A1* gene region was inversely associated with risk of thromboembolism (odds ratio per SD increase in estradiol 0.74; 95% CI, 0.62-0.90) (see Figs. 1 and 2, and Supplementary Tables 1, 4, and 5) (22). No excess heterogeneity was observed among the *CYP19A1* genetic variants in their associations with thromboembolism (see Supplementary Table 4) (22). Sensitivity analyses using Egger regression, adjusted for correlation among genetic variants, revealed no significant directional pleiotropy and an estimated causal effect of very similar magnitude as observed using the IVW MR (0.75; 95% CI, 0.59-0.96) (see Supplementary Table 4) (22). In contrast, endogenous testosterone predicted by variants in the *JMJD1C* gene region was directly associated with risk of thromboembolism (odds ratio per SD increase in log-transformed testosterone 1.39; 95% CI, 1.12-1.72) (see Supplementary Tables 2 and 4) (22).

Exploratory subanalyses evaluating venous and arterial thromboembolism separately revealed that endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region was inversely associated with risk of venous thromboembolism (0.74; 95% CI, 0.61-0.90; N = 5475 cases), whereas less-powered association analyses with arterial thromboembolism did not reach statistical significance (0.82; 95% CI, 0.43-1.56; N = 446 cases) (Supplementary Tables 5-7) (22). Further age-stratified analyses revealed that the effect sizes of the causal associations between serum estradiol and thromboembolism were rather similar for men younger than (OR, 0.73; 95% CI, 0.56-0.94) or older than (OR, 0.77; 95% CI, 0.59-1.00) the median age (61 years) for thromboembolism cases.

Table 1. Characteristics of the study participants

	n = 170 593
Age, y	57.0 (8.1)
Weight, kg	86.2 (14.3)
Height, cm	175.9 (6.8)
BMI	27.8 (4.2)
Primary outcome	
Thromboembolism (n, %)	5815 (3.4)
Exploratory outcomes	
Venous thromboembolism (n, %)	5474 (3.2)
Arterial embolism and thrombosis (n, %)	446 (0.3)
Ischemic stroke (n, %)	1755 (1.0)
Myocardial infarction (n, %)	9310 (5.4)

Values are given as mean (SD) or n (%).
Abbreviation: BMI, body mass index.

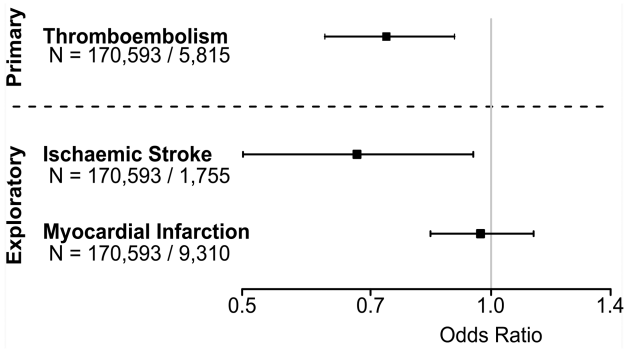

Figure 1. Estimated causal effects of endogenous estradiol genetically predicted by variants in the aromatase (*CYP19A1*) gene region on risk of the primary outcome thromboembolism and of the exploratory outcomes ischemic stroke and myocardial infarction. Odds ratios and 95% CIs for risk of disease are given per SD increase of estradiol. N, total number of participants/number of disease cases.

Endogenous Estradiol Was Inversely Causally Associated With Risk of Ischemic Stroke but not Myocardial Infarction

In further exploratory analyses, we investigated the associations of endogenous estradiol with potential repercussions of thromboembolism, namely ischemic stroke and myocardial infarction. Endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region was inversely associated with risk of ischemic stroke (0.68; 95% CI, 0.49-0.95) but not myocardial infarction

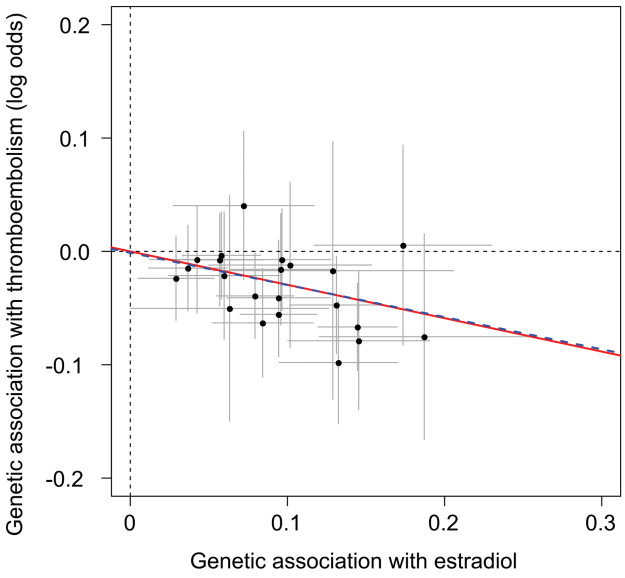

Figure 2. Genetic associations instrumented by genetic variants from the aromatase (*CYP19A1*) gene region with estradiol against thromboembolism. Genetic associations with estradiol (per allele increase in estradiol) were estimated in men only and taken from Eriksson et al (http://www.gefos.org/?q=content/estrogen-gwas-2018) (12). Genetic associations with thromboembolism (per allele log odds ratio) are estimated in men only in the UK Biobank. Associations for genetic variants and their CIs are represented by black dots with gray lines. The red solid line is the causal effect estimated by inverse variance–weighted mendelian randomization. The blue dashed line is the causal effect estimated using Egger regression.

(0.97; 95% CI, 0.84-1.13) (see Fig. 1 and Supplementary Tables 5-7) (22).

Discussion

Although an association was recently reported between genetic markers related to high testosterone and increased risk of thromboembolism, the impact of endogenous estradiol on the risk of thromboembolism in men was unclear. We show that, contrary to our hypothesis, endogenous estradiol genetically predicted by variants in the *CYP19A1* gene region was inversely associated with risk of thromboembolism in men in the UK Biobank. In the same setting, we replicated the previous finding that genetic variants in the *JMJD1C* gene region associated with high endogenous testosterone were associated with an increased risk of thromboembolism in men (10). Therefore, we suggest that the ratio between testosterone and estradiol, determined by aromatase activity, may play a role when evaluating the overall impact of sex steroids on thromboembolism in men. Subsequent explorative analyses evaluating potential repercussions of thromboembolism revealed that endogenous estradiol was inversely associated with risk of ischemic stroke but not myocardial infarction.

Previous observational association studies did not find any association between either endogenous testosterone (17, 18, 38) or estradiol (17, 18) and thromboembolism in men. The lack of consistent association in the observational studies could be explained by the heterogeneity in these studies and their susceptibility to residual confounding and reverse causality. In the present study, we replicated the recent finding that endogenous testosterone predicted by variants in the *JMJD1C* gene region was directly associated with risk of thromboembolism in men (10). However, the mechanism for the causal effect of testosterone on thromboembolism is unclear. As testosterone raises estrogen levels in men and it is well known that the use of oral contraceptives or hormone replacement therapy is associated with increased risk of thromboembolism in women (19, 20), while the risk associated with transdermal formulations is less (21), we hypothesized that estradiol might mediate the prothromboembolic effect of endogenous testosterone in men. Surprisingly, we observed that endogenous estradiol was robustly inversely associated with the risk of thromboembolism in men. Using MR, it has now been possible to estimate and separate some of the causal effects of endogenous testosterone and estradiol in men. The direct causal effects on bone mineral density and fracture risk were mainly exerted by endogenous estradiol with no or minimal contribution by endogenous testosterone (12, 13). In contrast, endogenous estradiol was indirectly causally associated with thromboembolism while genetic variants in the *JMJD1C* region, associated with high circulating testosterone, were associated with an increased risk of thromboembolism in men. Future studies are warranted to estimate and separate the causal effects of endogenous testosterone and estradiol on other major sex steroid–related phenotypes in men.

As endogenous estradiol was robustly associated with thromboembolism, we further investigated the associations of endogenous estradiol with potential repercussions of thromboembolism, namely ischemic stroke and myocardial infarction in men. We demonstrated that endogenous estradiol genetically predicted by variants in the *CYP19A1* region was inversely associated with risk of ischemic stroke but not with risk of myocardial infarction in men from the UK Biobank. In previous observational association studies, no consistent associations between serum estradiol and ischemic stroke or myocardial infarction have been reported in men (5, 6, 39, 40).

This study has a number of strengths. We employed state-of-the-art methods and our primary analysis, the causal association of endogenous estradiol with thromboembolism, was well powered to detect a moderate effect size. Also, we used a gene-based method in a biologically plausible gene to obtain unconfounded estimates with high precision using the cumulative unique information from multiple SNVs in the gene region explaining the majority of the identified variance in serum estradiol (10, 16). Another strength of the present study is that the genetic instruments and their effect estimates for serum estradiol were derived from the largest available GWAS meta-analysis in men not including the UK Biobank. Furthermore, for this GWAS, mass spectrometry–based analyses of serum estradiol were predominantly used because immunoassay-based techniques have a known questionable specificity, especially at the lower concentration range (26-29). In addition, the genetic associations with serum estradiol were estimated in different populations from the genetic associations with thromboembolism. The alleles of the genetic variants in the *CYP19A1* gene region that associate with increased serum estradiol do not associate with increased serum testosterone, enabling the differentiation between the causal effects of estradiol from those of testosterone (Fig. 3) (12, 13, 15). We also used both Egger regression and a *Q* statistic to exclude pleiotropic effects that might have confounded the results (37). Our analyses were, however, restricted to participants of White ancestry, so additional analyses are necessary to investigate whether our results also apply to those of other ethnicities. Another limitation of the present study is the fact that the validity of the *JMJD1C* region as a plausible source of instrumental variables to perform testosterone-related MR analyses has been questioned (41, 42). It has been suggested that there

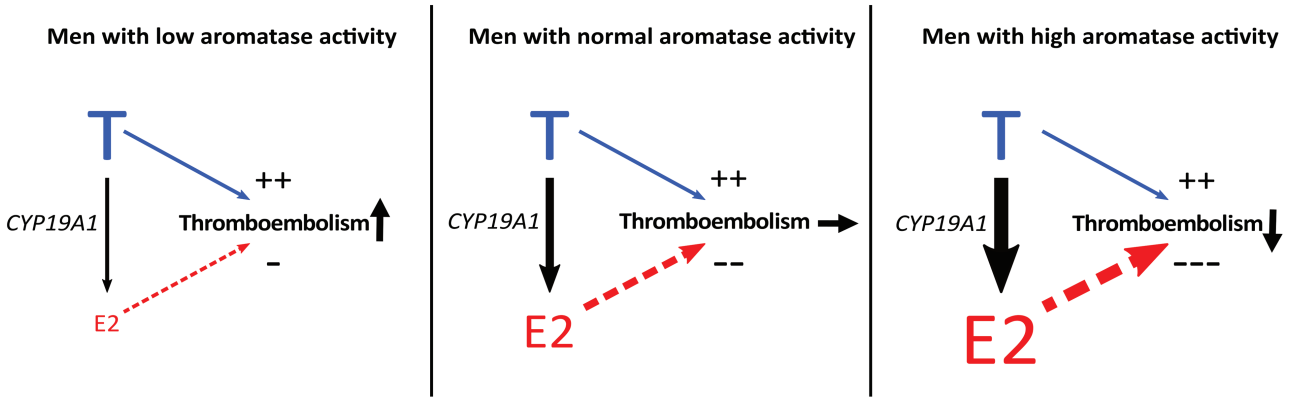

Figure 3. Proposed aromatase (*CYP19A1*)-dependent effects of serum sex steroids on risk of thromboembolism in men. Genetic variants in the *JMJD1C* gene region, associated with high circulating testosterone (T), were associated with increased risk of thromboembolism, while endogenous estradiol (E_2) was indirectly causally associated with thromboembolism. Based on these findings, we speculate that aromatase activity may contribute to determining the combined overall impact of serum sex steroids on risk of thromboembolism in men. Men with low aromatase activity (left panel) have relatively low serum E_2 and thereby the adverse effect of T is more prominent than the protective effect of E_2, resulting in an increased risk of thromboembolism. In contrast, men with high aromatase activity (right panel) have relatively high serum E_2 and thereby the beneficial effect of E_2 is more prominent than the adverse effect of T, resulting in a decreased risk of thromboembolism. There seems to exist a yin yang relationship between endogenous T and E_2 for risk of thromboembolism in men dependent on the activity of the aromatase enzyme.

is no clear physiological pathway by which the variants in the *JMJD1C* gene region may modulate the availability of testosterone and thus may affect the outcome via other traits than testosterone (41, 42). These possible horizontal pleiotropic effects may confound the analyses and potentially cause bias in the MR estimates. An alternative genetic instrument for testosterone may be sex hormone–binding globulin (*SHBG*) since its availability is known to modulate circulating testosterone concentrations. However, testosterone predicted by genetic variants in the *SHBG* gene region was not associated with thromboembolism (10). It should also be acknowledged that the present MR analyses are based on circulation estradiol levels. It is well known that estradiol is also synthetized and metabolized locally in tissues (43). The resulting local tissue levels may be important for local estradiol action but their potential causal effects on thromboembolism cannot be evaluated by the present MR approach.

We demonstrate that genetic variants in the *JMJD1C* gene region, associated with high circulating testosterone, were associated with an increased risk of thromboembolism while endogenous estradiol was indirectly causally associated with thromboembolism. Therefore, we speculate that the CYP19A1 (aromatase) activity, determining the relative balance between testosterone and estradiol, may contribute to assessing the combined impact of endogenous sex steroids on the risk of thromboembolism in men (see Fig. 3). In men with low aromatase activity, the adverse effects of endogenous testosterone will be more prominent, resulting in an increased risk for thromboembolism, whereas in men with high aromatase activity and thereby an efficient conversion of endogenous testosterone to estradiol, the protective effects of the latter will dominate, leading to a reduced risk of thromboembolism (see Fig. 3). Thus, there seems to exist a yin yang relationship between endogenous testosterone and estradiol for risk of thromboembolism in men that is dependent on the activity of the aromatase enzyme. These findings may have implications with respect to exogenous testosterone therapy in men with low testosterone. Future pharmacogenetic studies stratifying for genetically predicted aromatase activity are warranted to determine whether aromatase activity, and thereby the balance between testosterone and estradiol, affects the possible beneficial and adverse effects of testosterone replacement therapy on risk of thromboembolism and other sex steroid–related phenotypes. This lets us speculate that the aromatase enzyme, CYP19A1, may be a potential key enzyme for personalized medicine in men, as the testosterone dose may need to be individualized depending on the aromatase activity. Our findings may also have direct clinical implications for prostate cancer patients because the risk for thromboembolism of endocrine treatments affecting both endogenous estradiol and testosterone may differ compared to those modulating only androgen receptor action (44).

In conclusion, genetically predicted estradiol was inversely associated with the risk of thromboembolism and ischemic stroke in men. We speculate that the ratio between testosterone and estradiol, determined by aromatase activity, may contribute to evaluating the overall impact of sex steroids on thromboembolism in men. We propose that future pharmacogenetic studies stratifying for genetically predicted aromatase activity are warranted to determine whether the optimal safe testosterone dose for men with

low serum testosterone should be personalized depending on the aromatase activity.

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Author Contributions: Study design: M.N., J.Q., L.V., and C.O.; performing of analyses: M.N.; writing of original draft of the manuscript: M.N., L.V., and C.O. Interpretation of data, critical revision of the work, final version approval, and accountability for the work: all authors. Full access to all data in the study and responsibility for data integrity and accuracy of the data analysis: M.N. and C.O.

Additional Information

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Disclosures: The authors have nothing to disclose.

Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. Individual level data used to derive these results can be obtained with an approved application to the UK Biobank.

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