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Obesity as a causal risk factor for deep venous thrombosis: a Mendelian randomization study

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Abstract. Klovaite J, Benn M, Nordestgaard B (Herlev Hospital, Copenhagen University Hospital, Copenhagen; University of Copenhagen, Copenhagen; Gentofte Hospital, Copenhagen University Hospital, Hellerup; and Frederiksberg Hospital, Copenhagen University Hospital, Frederiksberg, Denmark). Obesity as a causal risk factor for deep venous thrombosis: a Mendelian randomization study. *J Intern Med* 2015; **277**: 573–584.

Objective. To test the hypothesis that obesity is causally associated with deep venous thrombosis (DVT).

Design. A Mendelian randomization design.

Setting. The Copenhagen General Population Study and the Copenhagen City Heart Study combined.

Subjects. Body mass index (BMI) measurements were available for 87 574 individuals of Danish descent from the adult general population. All subjects completed questionnaires and were genotyped for the *FTO* rs9939609 variant.

Main outcome measure. First events of DVT with or without pulmonary embolism (PE).

Analysis. The results were assessed using Cox regression, instrumental variable analysis and Poisson regression.

Results. Observationally, the risk of DVT increased with increasing BMI (*P*-trend < 0.0001). The multivariable-adjusted hazard ratio [95% confidence interval (CI)] for DVT was 1.3 (1.1–1.6) in overweight, 1.8 (1.4–2.2) in moderately obese and 3.4 (2.6–4.6) in severely obese compared with normal-weight individuals. For DVT complicated by PE, corresponding hazard ratios (95% CI) were 1.2 (0.8–1.8), 2.1 (1.3–3.5) and 5.1 (2.8–9.2). *FTO* AA versus TT genotype was associated with a 2.4% increase in BMI with hazard ratios (95% CI) of 1.09 (0.95–1.25) for DVT and 1.54 (1.12–2.10) for DVT complicated by PE. In instrumental variable analysis, the causal odds ratio (95% CI) for an increase in BMI of 1 kg m^{-2} was 1.13 (0.92–1.39) for DVT alone and 1.86 (1.14–3.02) for DVT complicated by PE. The absolute 10-year risk of DVT in a high-risk group (i.e. those aged >60 years and homozygous for Factor V Leiden) was 35% in obese individuals and 18% in normal-weight individuals.

Conclusion. A strong observational association between obesity and DVT with or without PE, supported by a direct genetic association between the obesity-specific locus *FTO* and DVT with PE, implies that obesity is likely to be causally associated with DVT.

Keywords: body mass index, deep venous thrombosis, general population, Mendelian randomization analysis.

Introduction

Obesity, measured as body mass index (BMI) >30 kg m^{-2}, has been associated in numerous studies with an approximate doubling of the risk of venous thromboembolism [1]. However, whether this represents a causal association is unknown.

Obesity is associated with inactivity, raised intra-abdominal pressure and decreased blood velocity in the legs, as well as with proinflammatory and prothrombotic states [2]. These effects are all likely to contribute to the risk of venous thromboembolism; that is, deep venous thrombosis (DVT) with or without complication by pulmonary embolism (PE). DVT complicated by PE is the most

severe form of venous thromboembolism and has a higher diagnostic correctness than DVT alone as it requires a medical diagnosis of both DVT and PE [3].

We used a Mendelian randomization approach to test the hypothesis that obesity is causally associated with increased risk of DVT with or without complication by PE. This method employs genetic variants associated with lifelong obesity and is analogous to a randomized clinical trial. Because genotypes are distributed randomly at conception, in the same way that drug or placebo is assigned randomly at study initiation in a randomized clinical trial, reverse caution and confounding are not important considerations. Therefore, both study types can be used to test whether or not exposures are causal. Prerequisites for a Mendelian randomization study include a strong genetic instrument without pleiotropic effects and sufficient statistical power.

Overall, 87 574 individuals of Danish descent from the Danish general population were followed for up to 35 years, and all DVT and PE events were recorded using the Danish national registries; BMI measurements were available for all participants and 86 267 were genotyped for the *FTO* rs9939609 polymorphism associated with lifelong increased BMI. The *FTO* (fat mass and obesity associated) gene was first described in 2007 as the gene most strongly associated with type 2 diabetes; however, the association disappeared after adjustment for BMI, indicating that the primary effect was due to BMI rather than to type 2 diabetes [4]. *FTO* encodes a protein most likely to be involved in fatty acid metabolism and energy homoeostasis, and it is thought to have a role in the hypothalamic regulation of appetite and food intake [5]. A large number of genes with very small effects each contribute to obesity, with *FTO* accounting for the largest proportion of variance (0.34%); therefore, variants within the *FTO* locus are particularly well suited for Mendelian randomization studies [5, 6].

Methods

Participants

All participants were white and of Danish descent and provided written informed consent. Of the 87 574 individuals included in the study with BMI measurements, genotyping data were available for 86 267.

The Copenhagen General Population Study

The Copenhagen General Population Study (CGPS) of the Danish general population was initiated in 2003 with ongoing enrolment, and end-points were ascertained from 1977 to 2011 [7]. Participants were randomly selected from the national Danish Civil Registration System to represent the general population of Danish descent, aged 20–100 years, resident in the greater Copenhagen area. Subjects were invited at random to participate in the study based on their Central Person Registration number (a unique identifier assigned to each individual in Denmark at birth or immigration). Of those invited, 45% agreed to participate.

On the day of attendance, data were obtained from a self-administered questionnaire reviewed by an examiner, blood samples were collected and participants underwent a physical examination. Blood samples for DNA extraction were available for the first 76 072 participants.

The Copenhagen City Heart Study

The Copenhagen City Heart Study (CCHS) of the Danish general population was initiated in 1976–1978, with follow-up examinations in 1981–1983, 1991–1994 and 2001–2003, and end-points were ascertained from 1977 to 2011 [8]. Participants were randomly selected from the national Danish Civil Registration System to represent the general population of Danish descent, aged from 20 to 100 years, resident in the City of Copenhagen. Participants were examined at study inclusion and again during follow-up exactly as in the CGPS. At each examination, the study population increased with new participants in younger age groups. Blood samples for DNA extraction were available for 10 195 participants attending the 1991–1994 and/or 2001–2003 examinations. Of those invited, 61% agreed to participate.

Outcome

All first DVT alone and DVT complicated by PE events were recorded. In both studies, data on diagnoses of DVT [according to World Health Organization International Classification of Diseases, eighth revision (ICD-8) codes 451.00, 451.08–09, 451.90, 451.92, 671.01–03 and 671.08–09 and 10th revision (ICD-10) codes I80.1–3, O22.3 and O87.1] and PE (ICD-8 codes 450.99 and 673.99 and ICD-10 codes I26.0, I26.9

and O88.2) were obtained from the national Danish Patient Registry (covering all contacts with public and private hospitals and including outpatients and emergency wards from 1995) and the national Danish Causes of Death Registry (reported by hospitals and general practitioners). These registries are 100% complete from 1977 onwards; therefore, not even a single individual from either study was lost to follow-up. A DVT diagnosis was based on ultrasonography or venography, whereas a PE diagnosis was based on ventilation/perfusion scintigraphy, ventilation/perfusion computed tomography and/or computerized tomographic pulmonary angiography. Registry-based diagnoses of venous thromboembolism in Denmark have previously been validated, with an overall positive predictive value of a discharge diagnosis of venous thromboembolism of 55–72% [9, 10].

Deep venous thrombosis alone was defined as DVT with no record of PE. DVT complicated by PE was assessed in parallel in an attempt to reduce the possibility of potential misclassification of DVT diagnosis; that is, a PE diagnosis was used to identify cases of DVT with a higher diagnostic correctness than DVT alone. Individuals with isolated PE were excluded from the analysis because it is possible that isolated PE may be caused by factors other than thrombi from DVT, such as fat or cancer cell emboli, which are not specified in the Danish Patient Registry or the Danish Causes of Death Registry. Unprovoked events were defined as events in patients without major clinical risk factors such as a cancer diagnosis within 1 year before and after the event (data from the national Danish Cancer Registry started in 1943), without major surgery within 3 months before the event (data from the national Danish Patient Registry) and without a Factor V Leiden *F5* (rs6025) mutation (i.e. the most important genetic risk factor for DVT and PE).

Body mass index

Body mass index was calculated as body weight in kilograms divided by height in metres squared (kg m^{-2}).

Genotypes

Genotyping for *FTO* (rs9939609) and Factor V Leiden *F5* (rs6025) was performed using TaqMan assays and ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA); Factor V Leiden was used for adjustments and stratifications. Each run included a known noncarrier, as well as a heterozygous and a homozygous control for each genotype. After two reruns, call rates for genotypes were above 99.96% for both assays and both were in Hardy–Weinberg equilibrium. Genotyping was verified by DNA sequencing in 16 randomly selected individuals for each genotype. The study populations were also genotyped for two other variants reported to be associated with BMI, albeit less strongly than *FTO*: *MC4R* (rs17782313) and *TMEM18* (rs6548238) [5, 11, 12]. These genotypes did not show statistically significant association with DVT and/or PE (Figures S1 and S2) and were excluded from further analysis.

Other covariates

Smoking (current and/or former), use of lipid-lowering therapy, oral contraceptives and hormone-replacement therapy, menopausal status and level of physical activity were self-reported. Recent major surgery was defined as any surgical procedure requiring general anaesthesia within 3 months before the event.

Statistical analysis

We tested the hypothesis that obesity is causally associated with increased risk of DVT, with or without complication by PE. We used STATA 12 (Stata Corp., College Station, TX, USA) for all statistical analyses.

Participants were divided based on standard BMI categories defined by the National Heart Lung and Blood Institute as follows: underweight (<18.5 kg m^{-2}), normal weight (18.5–24.9 kg m^{-2}), overweight (25.0–29.9 kg m^{-2}) and obese (≥30.0 kg m^{-2}) [13]; obese participants were additionally subdivided into those who were moderately (30.0–34.9 kg m^{-2}) or severely obese (≥35 kg m^{-2}). Underweight individuals (n = 806) were excluded from the analysis as this low level of BMI may be the consequence of severe illness.

Hazard ratios with 95% confidence intervals (CIs) were calculated using Cox regression models with age as the time scale (i.e. age is automatically adjusted for, referred to below as age adjustment) and left truncation (delayed entry) after adjustment for (i) age and gender and (ii) multivariable

adjustment for risk factors of DVT and potential confounding factors (i.e. age, gender, smoking, physical activity, use of lipid-lowering therapy, Factor V Leiden, cancer, recent major surgery and study, and for woman only additionally for use of oral contraceptives, use of hormone-replacement therapy and menopausal status). We used stratified analyses to compare the risk between obese (BMI ≥30 kg m^{-2}) and normal-weight individuals (BMI 18.5–24.9 kg m^{-2}) in groups of all covariates as well as in the two studies separately and performed tests of interaction between BMI categories and all covariates included in multivariable adjustment, and for study. In stratified analyses, we corrected for multiple comparisons using the Bonferroni method [*P*-values were multiplied by 26 individual tests (13 stratifications × 2 endpoints)]. The effect of interaction between two covariates on risk of disease was evaluated using a likelihood ratio test. Missing values (<1%) for covariates were imputed using multivariable normal regression imputation (Stata command 'mi impute mvn'), where age and BMI at examination, and gender, were independent variables; smoking and physical activity were dependent variables in the model. Individuals with missing Factor V Leiden genotypes (24%) were categorized as missing for the calculation of risk estimates.

We used a Mendelian randomization approach to investigate a potential causal relationship between elevated BMI and DVT. First, using Cox regression, we tested the observational association described previously. Secondly, using analysis of variance, we evaluated whether genotypes were associated with BMI and calculated per allele effects of genotypes in the study population. We used a single nucleotide polymorphism (SNP) in the *FTO* locus (rs9939609*)*. This locus has been identified in genomewide association studies as having the largest effect on BMI [5, 6, 14, 15]. Thirdly, to test whether genetically elevated BMI is associated with increased risk of DVT, we tested for association between genotypes and DVT risk with and without complication by PE using Cox regression. These analyses were only adjusted for age as, according to the principle of Mendelian randomization, covariates were randomly distributed across genotypes. Finally, we performed an instrumental variable analysis using the control function estimator [16, 17] to obtain an estimate of the potential causal effect of lifelong increased BMI on risk of DVT. The control function estimator method comprises two regression stages, using the residuals from the first stage in the second-stage regression, incorporating information from confounders. As an instrument we used the obesity-specific locus *FTO* rs9939609, the strength of which was evaluated by *F*-statistics, where $F > 10$ indicated a strong statistical instrument of the instrumental variable analysis, while R^2 as a percentage is a measure of the contribution of genotype to the variation in BMI [18]. Based on this regression, we calculated a causal odds ratio for DVT and DVT complicated by PE for a genetically induced increase in BMI of 1 kg m^{-2}. As BMI and residuals were slightly non-normally distributed, instrumental variable analysis were also performed with BMI log (ln) transformed to approach normal distribution; similar results were obtained. We also performed a test for endogeneity to examine differences between observational and instrumental variable models, to test for the effect of unknown confounders in the observational model [19].

Absolute 10 year risks of DVT (with and without PE combined) by categories of BMI, Factor V Leiden noncarriers/heterozygotes/homozygotes and by age (<40, 40–60 and >60 years) were estimated using the regression coefficients from a Poisson regression model; these three covariates were the most influential factors in determining 10-year risk of DVT, whereas the influence of other factors (e.g. smoking) was minimal. Absolute risks are presented as estimated incidence rates (events/10 years) in percentage.

Results

Characteristics of participants by BMI categories are shown in Table 1. Participants were followed for up to 35 years for DVT and PE. From 1977 to 2011, DVT was recorded in 2158 participants; of these, DVT complicated by PE was observed in 299 individuals. The CGPS and the CCHS cohorts were combined to obtain maximal power; however, the results were similar within each study separately.

BMI and DVT: observational estimates

The risk of DVT increased with increasing BMI (Fig. 1; all *P*-trend < 0.0001). Compared with normal weight individuals (BMI 18.5–24.9 kg m^{-2}), after multivariable adjustment for age, gender, smoking, physical activity, use of lipid-lowering therapy, Factor V Leiden, history of cancer, recent major surgery and study, and for women only additionally for use of oral contraceptives, use of

Table 1 *Characteristics of 87 574 individuals from the general population of Danish descent*

	Normal weight	Overweight	Moderately obese	Severely obese
BMI, mean (min–max), kg m^{-2}	22.7 (18.5–24.9)	27.2 (25.0–29.9)	31.9 (30.0–34.9)	38.4 (35.0—64.8)
Participants, *n*	38 421	34 821	11 088	3244
Women, %	65	45	49	65
Age, median (IQR), years	55 (65–45)	59 (68–49)	60 (68–51)	58 (66–49)
Deep venous thrombosis (%)[a]	542 (1)	794 (2)	369 (3)	154 (5)
Unprovoked (%)[b]	304 (56)	459 (58)	213 (58)	88 (57)
Deep venous thrombosis and pulmonary embolism (%)[a]	84 (0.2)	124 (0.4)	56 (0.5)	35 (1.0)
Unprovoked (%)[b]	45 (54)	77 (62)	29 (52)	20 (57)
Any cancer, %[c]	9	10	11	9
Major surgery, %[d]	14	13	14	15
Factor V Leiden *F5* (rs6025) carriers, %[e]	9	8	9	8
Ever smokers, %	60	63	63	61
Low physical activity, %	39	44	52	63
Lipid-lowering therapy, %	6	12	16	17
Oral contraception use, %[f]	7	4	4	4
Hormone-replacement therapy, %[f]	13	13	11	8
Menopause, %[f]	63	77	79	75

BMI, body mass index; IQR, interquartile range.
[a]Number of first events at the end of follow-up (percentage of all participants in this BMI category). [b]Number of events in individuals without recent major surgery (within 3 months before the event), cancer diagnosis (1 year before or after event) or Factor V Leiden *F5* (rs6025) mutation (percentage of deep venous thrombosis with or without pulmonary embolism in this BMI category). [c]Cancer diagnosis within 1 year before or after event. [d]Major surgery within 3 months before the event. [e]Carriers were heterozygous and homozygous combined. [f]Women only.

hormone-replacement therapy and menopausal status, the hazard ratios (95% CIs) were 1.3 (1.1–1.6) in overweight individuals (BMI 25.0–29.9 kg m^{-2}), 1.8 (1.4–2.2) in moderately obese individuals (BMI 30.0–34.9 kg m^{-2}) and 3.4 (2.6–4.6) in severely obese individuals (BMI ≥35 kg m^{-2}; Fig. 1, upper right panel). For the end-point DVT complicated by PE, the corresponding hazard ratios (95% CIs) were 1.2 (0.8–1.8), 2.1 (1.3–3.5) and 5.1 (2.8–9.2; Fig. 1, lower right panel).

In stratified analyses, the risks of DVT alone and of DVT complicated by PE were increased in obese (BMI ≥30 kg m^{-2}) versus normal-weight individuals (BMI 18.5–24.9 kg m^{-2}) in all strata of all covariates as well as in the two studies separately (Fig. 2). The relatively few strata with 95% CIs overlapping 1.0 were typically those with the fewest number of events, suggesting that the lack of significance was due to low statistical power in the specific strata. Accordingly, tests of interaction between BMI categories and other covariates including study showed no evidence of interaction for any stratification.

BMI levels by genotype

FTO (rs9939609) AA versus TT genotype was associated with increases in BMI of 2.4% (95% CI 2.1–2.7%) corresponding to 0.6 kg m^{-2} (Fig. 3, left panel). This genotype explained 0.3% (R^2) of the variation in BMI. The associations between *MC4R* (rs17782313) and *TMEM18* (rs6548238) genotypes and BMI are presented in Figures S1 and S2 (left panels).

Genotype and DVT

Because *FTO* genotype is unrelated to potential confounding factors (Table S1), and DVT and PE cannot alter genotypes, an association between BMI-increasing alleles and these disorders would indicate a causal association. Indeed, in relation to the strongest end-point, we observed an

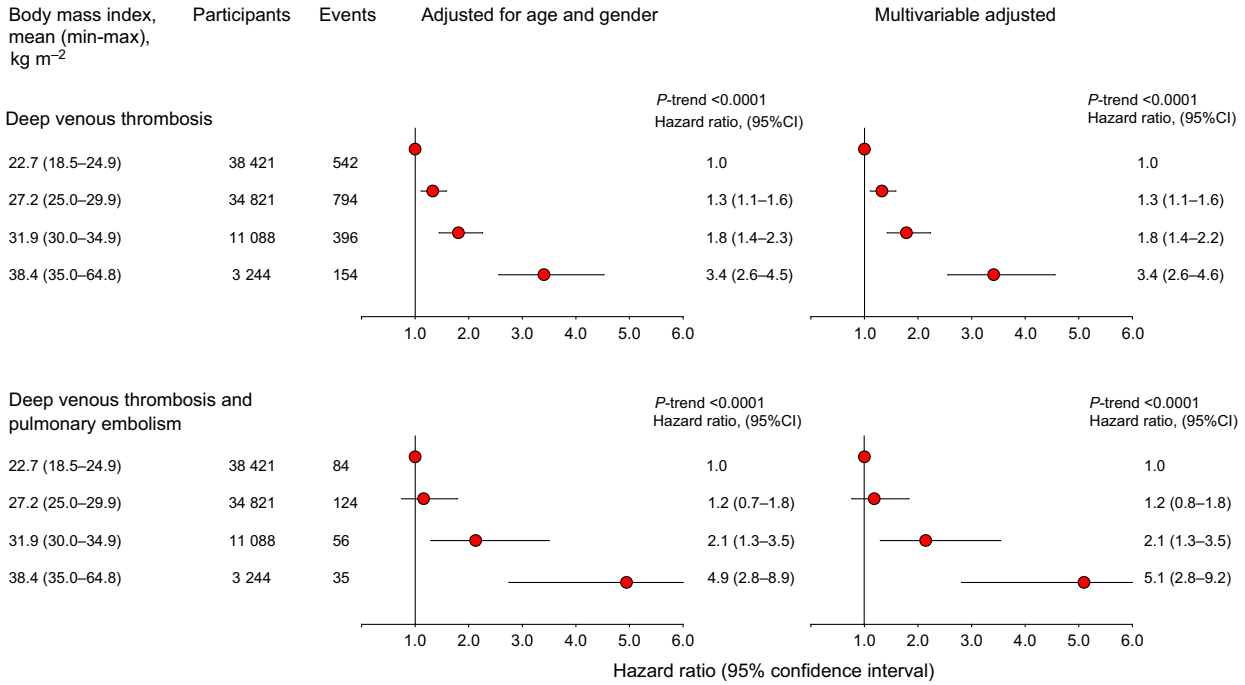

Fig. 1 *Risk of deep venous thrombosis alone and complicated by pulmonary embolism by categories of body mass index (BMI) in the general population of Danish descent. Data are presented as hazard ratios with 95% confidence intervals [(CIs); black dots and horizontal lines], adjusted for age and gender, or following multivariable adjustment for age, gender, smoking, physical activity, use of lipid-lowering therapy, Factor V Leiden genotype, history of cancer, recent major surgery and study, and for woman only additionally for use of oral contraceptives, use of hormone-replacement therapy and menopausal status. The risk was assessed in the Copenhagen General Population Study and the Copenhagen City Heart Study combined in 87 574 individuals followed from 1977 to 2011. Individuals in whom events occurred before examination were excluded from the analysis. P-values are given for trend across BMI categories as normal weight (18.5–24.9 kg m^{-2}), overweight (25.0–29.9 kg m^{-2}), moderately obese (30.0–34.9 kg m^{-2}) and severely obese (≥35 kg m^{-2}).*

association between *FTO* AA versus TT genotype and DVT complicated by PE with a hazard ratio of 1.54 (95% CI 1.12–2.10; Fig. 3, right panel; *P*-trend = 0.02). No statistically significant association was observed between *FTO* genotype and the weaker end-point of DVT alone (Fig. 3, middle panel; *P*-trend = 0.2); however, the hazard ratio for *FTO* AA versus TT genotype of 1.09 (95% CI 0.95–1.25) was nominally above 1.0. The associations between *MC4R* (rs17782313) and *TMEM18* (rs6548238) genotypes and both DVT and DVT with PE are presented in Figures S1 and S2 (right panels).

BMI and DVT: causal estimates

Instrumental variable analysis indicated sufficient statistical strength of the *FTO* genetic variant as an instruments ($F = 118$). An increase in BMI of 1 kg m^{-2} due to *FTO* genotype was associated with a causal odds ratios for DVT of 1.13 (95% CI 0.92–1.39) with a corresponding observational multivariable adjusted odds ratio of 1.08 (95% CI 1.07–1.09; *P* for endogeneity = 0.7; Fig. 4). The corresponding values for DVT complicated by PE were 1.86 (95% CI 1.14–3.02) and 1.09 (95% CI 1.06–1.12; *P* for endogeneity = 0.03).

Absolute risk of DVT

We observed a rise in absolute 10-year risk of DVT (with and without PE combined) with increasing BMI, number of Factor V Leiden alleles and age (Fig. 5). An absolute 10-year risk of DVT of 35% was found in obese high-risk individuals (i.e. those aged >60 years and homozygous for Factor V Leiden). By comparison, normal-weight high-risk individuals in the same category had an absolute 10-year risk of DVT of 18%.

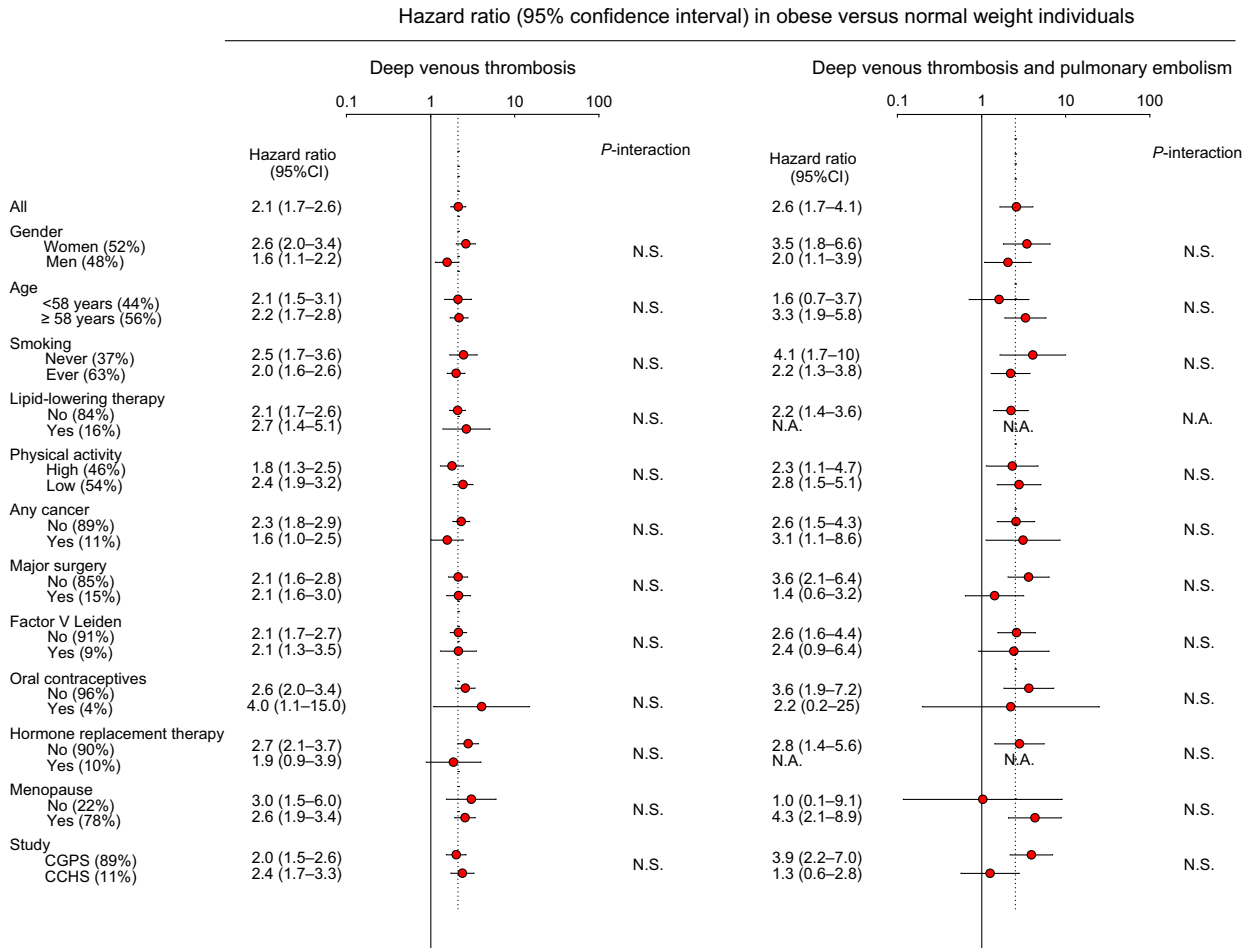

Fig. 2 *Risk of deep venous thrombosis alone and complicated by pulmonary embolism for obese versus normal-weight individuals. Data are presented as hazard ratios with 95% confidence intervals [(CIs); black dots and horizontal lines], following multivariable adjustment for age, gender, smoking, physical activity, use of lipid-lowering therapy, Factor V Leiden genotype, history of cancer, recent major surgery and study, and for woman only additionally for use of oral contraceptives, use of hormone-replacement therapy and menopausal status. Individuals in whom events occurred before examination were excluded from the analysis. P-values are given for test of interaction between obese (body mass index ≥30 kg m^{-2}) versus normal-weight (body mass index 18.5–24.9 kg m^{-2}) individuals and covariates with Bonferroni correction: P-values were multiplied by 26 individual tests (13 covariates × 2 end-points). N.S., not significant (original P-value multiplied by 26 to account for multiple comparisons > 1.0); N.A., not available; CGPS, Copenhagen General Population Study; CCHS, Copenhagen City Heart Study.*

Discussion

A novel finding of this study is that the obesity-specific locus *FTO* rs9939609 was associated with increased risk of DVT complicated by PE, an end-point less likely to be prone to misclassification than DVT alone.

A strong independent observational association between obesity and DVT with or without PE, supported by the genetic association between the obesity-specific *FTO* locus and DVT with PE, implies that obesity is likely to be causally associated with DVT. The results of instrumental variable analysis further support our hypothesis, suggesting that increased risk of DVT with or without PE is more likely to be due to obesity *per se* than mediated through other cardiovascular disease risk factors. This is an important finding which suggests that treating risk factors associated with

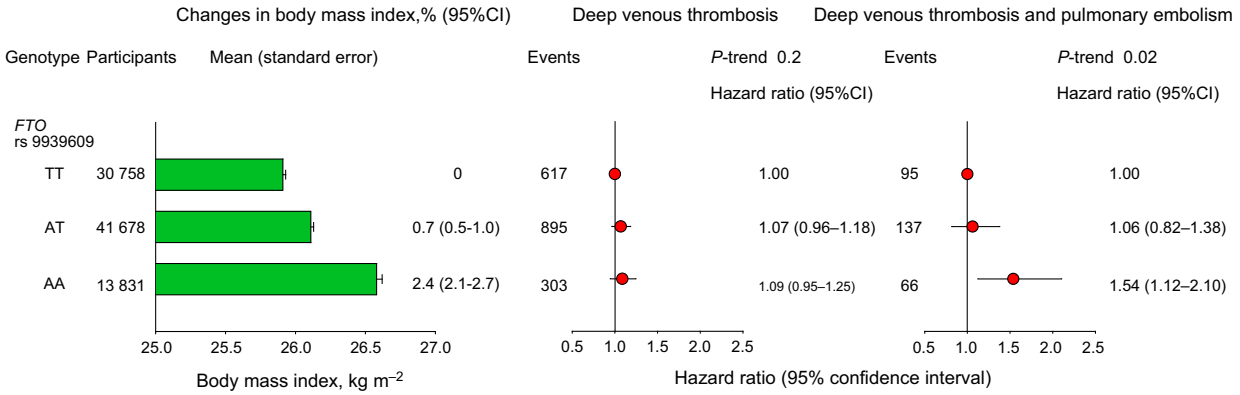

Fig. 3 *Risk of deep venous thrombosis alone and complicated by pulmonary embolism as a function of FTO genotype. A total of 86 267 participants of the Copenhagen General Population Study and the Copenhagen City Heart Study (combined to obtain maximum statistical power) were genotyped; body mass index measurements were available for 86 112 of these individuals. Hazard ratios were only adjusted for age, as genotype was not associated with potential confounding factors (see Table S1). P-values represent trend across genotypes.*

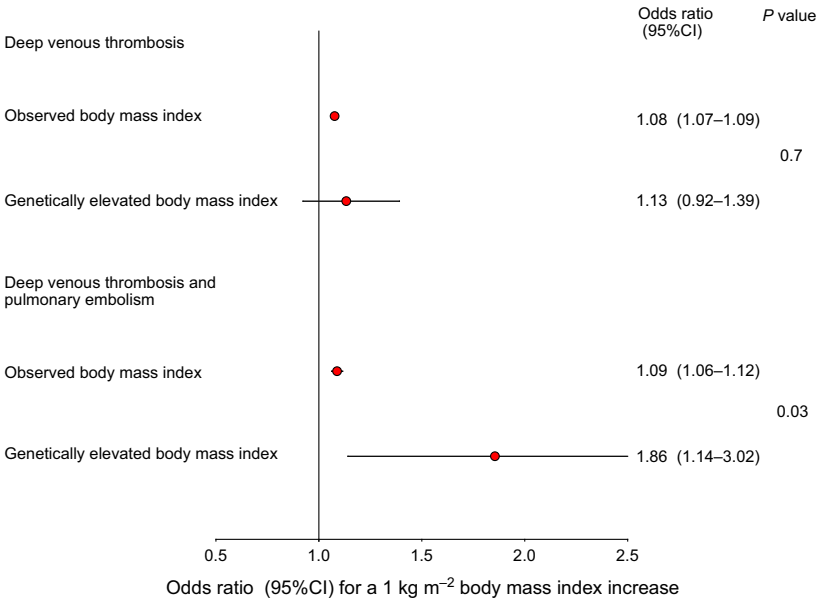

Fig. 4 *Summary of observational and causal associations between increased body mass index and increased risk of deep venous thrombosis alone and complicated by pulmonary embolism. The comparison between observationally multivariable-adjusted and genetically elevated body mass index was performed in the Copenhagen General Population Study and the Copenhagen City Heart Study (combined to obtain maximum statistical power). The effect of genetically elevated body mass index on risk of deep venous thrombosis alone and complicated by pulmonary embolism was estimated by the association between a genetic increase of 1 kg m^{-2} in body mass index and risk of deep venous thrombosis alone and complicated by pulmonary embolism, using the control function estimator method for instrumental variable analysis with results presented as odds ratio with 95% confidence interval (CI). P-values are given for endogeneity test.*

obesity will not be sufficient to eliminate the risk of DVT/PE in obese individuals. In addition, we found that the association between increased BMI and increased risk of DVT is higher than previously reported (up to fivefold versus twofold increased) and that this association is not attenuated after

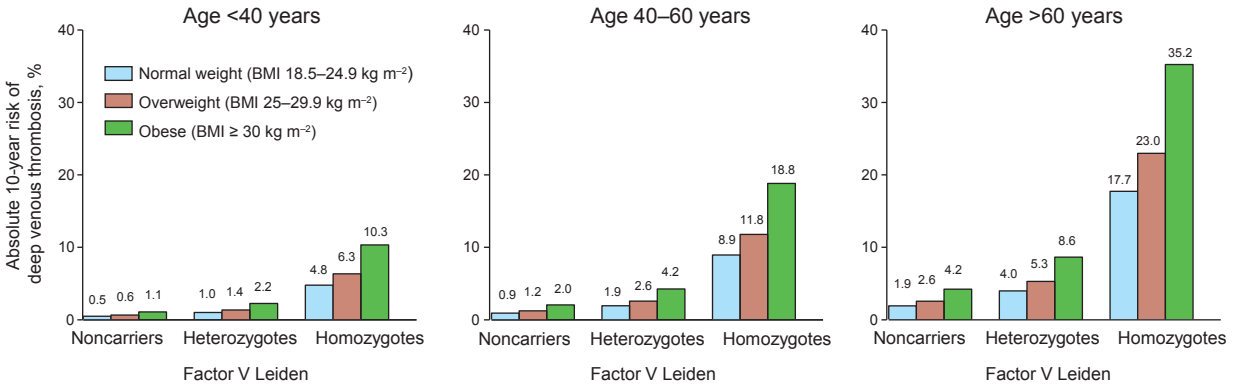

Fig. 5 *The absolute 10-year risk of deep venous thrombosis by body mass index categories, age and Factor V Leiden genotype. Absolute 10-year risk of deep venous thrombosis (with or without pulmonary embolism combined) by categories of body mass index, Factor V Leiden non-carriers/heterozygotes/homozygotes and age (<40, 40–60 and >60 years) were estimated using regression coefficients from a Poisson regression model. Absolute risks are presented as estimated incidence rates (events/10 years) in percentage. Data based on 87 574 individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study are combined to obtain maximum statistical power.*

extensive multivariable adjustment. For use in clinical practice, we calculated absolute 10-year risk of DVT as a function of BMI, age and Factor V Leiden genotype.

Mechanistically, there are several pathways that could underlie a causal relationship between obesity and increased risk of DVT [2, 20]. Pathophysiologically, an increase in fat mass at all stages of life leads to inactivity, higher intra-abdominal pressure and slower blood circulation in the lower limbs, creating a prothrombotic milieu. Furthermore, adipose tissue is not only a storage site for excess energy, but also a multifunctional organ that secretes numerous hormones and cytokines, thus influencing cell functions and gene expression [2, 20]. As a consequence of overnutrition, adipose tissue undergoes remodelling: adipocytes grow in size and number, accompanied by altered secretion, angiogenesis and inflammation. Adipocytes are stimulated to secrete tissue factor (the trigger for blood coagulation), plasminogen activator inhibitor (which impairs fibrinolytic function) and substances responsible for local vessel degradation, all of which may promote clot initiation and formation and increase the risk of developing DVT [2, 20].

In support of our findings, obesity has consistently been shown to be a risk factor for venous thromboembolism [1, 21, 22]. A pooled analysis of one cohort and eight case–control studies with a total of 8125 thrombosis patients and 23 272 control subjects demonstrated an odds ratio of venous thromboembolism of 2.3 (95% CI 1.7–2.3) for BMI >30 kg m^{-2} versus ≤30 kg m^{-2} [1]. Similar results were obtained from studies assessing the risk of DVT specifically [23, 24]. In addition, several studies have demonstrated that obesity is associated with increased risk of PE [25, 26]; however, taking into account the fact that thrombotic PE is a consequence of DVT, these results may imply an underlying risk of DVT rather than of PE.

As expected, we observed a strong association between obesity and DVT with and without PE. The risk of DVT appeared to be increased up to fivefold in severely obese individuals (BMI ≥35 kg m^{-2}) compared with those of normal weight (BMI 18.5–24.9 kg m^{-2}). These risk estimates are larger than the twofold increased risk observed in previous studies, probably because we excluded underweight individuals with a BMI below 18.5 kg m^{-2}; such low BMI values may influence the reference group in analyses as some individuals with very low BMI values due to severe illness would be included in this group [27, 28]. In addition, we subdivided the standard category for obesity into moderate and severe obesity, to avoid underestimating the risk of DVT for severely obese individuals. Furthermore, the fact that we used the additional end-point of DVT complicated by PE and excluded individuals with isolated PE allowed us to eliminate possible DVT and PE misdiagnoses and

to confirm that the association between BMI and DVT is more pronounced than previously reported. We did not assess isolated PE as an end-point, because of limited statistical power and because the existence of isolated PE as an entity is highly questionable [29]. To the best of our knowledge, PE is always preceded by DVT, unless the embolus derives from the right atrium during atrial fibrillation or is not of thrombotic origin. Furthermore, there is no evidence to suggest that obesity would promote PE rather than DVT.

It is unclear whether obesity is truly an independent risk factor for DVT, or whether the effect is secondary to diabetes and dyslipidaemia [30]. The present study is the first to evaluate a potential causality between obesity and DVT using a Mendelian randomization approach. Mendelian randomization uses analyses analogous to those in a randomized trial, but randomization at conception in this case refers to that for genotypes associated with the risk factor BMI [18, 31, 32]. We used the *FTO* rs9939609 polymorphism, which has been reliably shown to be associated with BMI [4, 14, 15], as a suitable proxy for lifelong elevated BMI, free of confounding and reverse causation. In the present study, the *FTO* genotype was associated with an increased risk of DVT complicated by PE. Even if *FTO* genotype explains only a small part of the variation in BMI, in instrumental variable analysis, it had enough strength as an instrument to demonstrate that a genetic increase in BMI of 1 kg m^{-2} had a statistically significant effect on risk of DVT complicated by PE, supporting our hypothesis that obesity is causally related to DVT. Results of the instrumental variable analysis regarding DVT alone were also compatible with causality, as there was no endogeneity problem in the observational model ($P = 0.7$), that is, the observational model in this case can be used as a proxy causality estimate. Technically, endogeneity effects occur when there is correlation between an independent (causal) variable and an error term (e.g. unknown omitted variables, unobserved heterogeneity or reverse causality) in the statistical model that is not easy to detect or control [33]. The instrumental variable technique is used to counteract endogeneity issues, thus offering a way to overcome unobserved confounding in observational studies and make causal inferences. To evaluate whether the observational or instrumental variable technique is the best method in a given circumstance, we tested for endogeneity using the control function approach [17, 19]. Under the null hypothesis, estimates from both models are equal, and therefore, there is no problem of endogeneity and estimates from the observational rather than the instrumental variable model can be used. As mentioned above, this was the case for the end-point DVT, and thus, the observational estimate in the DVT model and the instrumental variable estimate in DVT with PE model are both indicative of causality.

Our calculations of 10-year absolute risk of DVT could be directly implemented as a clinical tool for advising individual patients in a clinical setting. The association between increased BMI and increased risk of DVT, irrespective of age and Factor V Leiden genotype, suggests that more intensive efforts to reduce obesity, a modifiable risk factor for DVT, may be warranted, particularly because both nongenetically and genetically determined body fat excess can be altered by reducing food intake and by increasing physical activity [34–36].

The main strength of the present study is the large sample size followed for up to 35 years; this meant that we had the statistical power even to document increased risk of DVT complicated by PE as a function of BMI-increasing genotype, using a single genetic variant with a relatively small effect as an instrumental variable. A further strength is the fact that all participants were white and of Danish descent, thus excluding confounding due to population admixture, which can be a major problem in multiracial genetic studies.

A potential limitation of our study is that end-points were obtained from computerized registries and have not been individually validated. However, we used the diagnosis of PE to confirm our primary end-point, DVT, and thus to obtain a stronger end-point than DVT alone. Furthermore, we have previously validated the present data by evaluating risk of DVT alone and with PE in carriers of the Factor V Leiden mutation *F5* (rs6025), in the same populations as in the present study and using similar methods [7]. As participation rates were 45% and 61% in the two studies, it is possible that less healthy and obese individuals were less likely to participate compared with healthy individuals of normal weight. Such a potential bias would probably be nondifferential, particularly for *FTO* genotype and therefore is unlikely to explain our

results. A further limitation is that we were not able to demonstrate a statistically significant association between two other BMI-associated loci, *MC4R* (rs17782313) and *TMEM18* (rs6548238) [11, 12], and risk of DVT alone or complicated by PE; however, these genotypes have been shown to have even less effect on BMI than the *FTO* genotype and therefore less statistical power. Nevertheless, we were able to detect a trend towards an association between the *MC4R* variant and DVT with PE, especially using risk allele score combined with the *FTO* genotype. The contribution of the *MC4R* polymorphism to total variation in fat mass is smaller than that of *FTO*, explaining only 0.14% compared with 0.34% explained by *FTO* [6, 14, 37]. The genetic polymorphism rs6548238 close to the *TMEM18* gene showed a trend towards a negative association with DVT complicated by PE, possibly because of the low overall frequency of its nonrisk allele. Furthermore, the role of *TMEM18* in body weight is very poorly understood. All three described genetic variants are located in or near genes widely expressed in the central nervous system [5]. In contrast to *FTO* and *MC4R*, as demonstrated in earlier studies, there is no regulation of *TMEM18* in the hypothalamus or brainstem during food deprivation or consumption of a high-fat diet [38]. Additionally, *TMEM18* (rs6548238) has a genetic effect on diabetes even after adjustment for BMI [39], indicating that its role in the risk of diabetes is not mediated through modulation of adiposity. Therefore, it seems plausible that *TMEM18* could affect DVT with PE not only through increased BMI, but also by other unknown biological pathways, possibly explaining the unexpected findings for this genotype.

It could also be argued that inclusion of only white subjects of Danish descent is a limitation of this study; however, we are not aware of any evidence to suggest that our findings should not be applicable to most races in most countries.

There are a number of potential limitations of Mendelian randomization studies, as discussed elsewhere [18, 31, 32]; however, these are unlikely to explain the results of the present study [11]. Furthermore, instrumental variable analysis presents statistical challenges when the outcome variable is binary and the phenotype continuous. The control function estimator, or adjusted two-stage odds ratios method, gives a valid test of the null hypothesis, but interpretation and comparison of the magnitude of odds ratios are limited [16]. However, the main focus of a Mendelian randomization study is identifying causal risk factors, not precise estimation of causal effects [16].

In conclusion, a strong observational association between obesity and DVT with or without PE, supported by a direct genetic association between the obesity-specific locus *FTO* and DVT with PE, implies that obesity is most likely to be causally associated with DVT. This finding suggests that the incidence of DVT will rise with the growing epidemic of obesity, and hence improved preventive strategies and early recognition of DVT in obese individuals are of critical importance.

Conflict of interest statement

No conflict of interest was declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of individuals from the Danish General Population Study by genotype.

Figure S1. Risk of deep venous thrombosis alone and complicated by pulmonary embolism as a function of *FTO* and *MC4R* genotypes and allele score.

Figure S2. Risk of deep venous thrombosis alone and complicated by pulmonary embolism as a function of *FTO, MC4R* and *TMEM18* genotypes and allele score.