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Clinical and Genetic Determinants of Varicose Veins

Prospective, Community-Based Study of ≈500 000 Individuals

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BACKGROUND: Varicose veins are a common problem with no approved medical therapies. Although it is believed that varicose vein pathogenesis is multifactorial, there is limited understanding of the genetic and environmental factors that contribute to their formation. Large-scale studies of risk factors for varicose veins may highlight important aspects of pathophysiology and identify groups at increased risk for disease.

METHODS: We applied machine learning to agnostically search for risk factors of varicose veins in 493 519 individuals in the UK Biobank. Predictors were further studied with univariable and multivariable Cox regression analyses (2441 incident events). A genome-wide association study of varicose veins was also performed among 337 536 unrelated individuals (9577 cases) of white British descent, followed by expression quantitative loci and pathway analyses. Because height emerged as a new candidate risk factor, we performed mendelian randomization analyses to assess a potential causal role for height in varicose vein development.

RESULTS: Machine learning confirmed several known (age, sex, obesity, pregnancy, history of deep vein thrombosis) and identified several new risk factors for varicose vein disease, including height. After adjustment for traditional risk factors in Cox regression, greater height remained independently associated with varicose veins (hazard ratio for upper versus lower quartile, 1.74; 95% CI, 1.51–2.01; $P<0.0001$). A genome-wide association study identified 30 new genome-wide significant loci, identifying pathways involved in vascular development and skeletal/limb biology. Mendelian randomization analysis provided evidence that increased height is causally related to varicose veins (inverse-variance weighted: odds ratio, 1.26; $P=2.07\times10^{-16}$).

CONCLUSIONS: Using data from nearly a half-million individuals, we present a comprehensive genetic and epidemiological study of varicose veins. We identified novel clinical and genetic risk factors that provide pathophysiological insights and could help future improvements of treatment of varicose vein disease.

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Clinical Perspective

What Is New?

- In this population-based study of ≈500 000 individuals, greater height appeared as a novel predictor of varicose vein disease in machine learning analyses and was independently associated in multivariable-adjusted Cox regression.
- Using mendelian randomization, we demonstrate that greater height has a causal role in varicose vein development.
- We identify 30 genetic loci in the first large-scale genome-wide analysis of varicose veins, including 337 536 individuals (9577 cases and 327 959 controls), and discover a strong genetic correlation between varicose veins and deep vein thrombosis.

What Are the Clinical Implications?

- Varicose veins are a common condition that are increasingly being associated with serious health risks such as venous thromboembolism, yet little is known about the genetic and environmental risk factors for varicose vein disease.
- Our study demonstrates the causal role of height and identifies novel genetic and clinical associations with varicose veins.
- This knowledge greatly expands our understanding of disease pathophysiology and may help future improvements in the management of varicose veins and their associated complications.

Varicose veins are a common manifestation of chronic venous disease. It is estimated that >30 million adults in the United States have varicose veins, with interventions consuming more than $1 billion in direct healthcare resources per year.[1] Although sometimes dismissed as a cosmetic finding, varicose veins can culminate with life-limiting ulceration in up to 20% of affected individuals and have increasingly been associated with serious health risks.[2] Recent studies show that patients with varicose veins have up to a 5-fold increased risk for developing deep vein thrombosis (DVT).[3,4] Moreover, the association of varicose veins with peripheral artery disease (PAD) and additional vascular diseases is also being elucidated.[3,5]

The factors influencing varicose vein formation are not fully understood. Epidemiological studies have established multiple risk factors such as age, female sex, pregnancy, obesity, and prior DVT,[6–8] but several other putative factors remain unconfirmed. There is also a clear familial component to varicose vein disease, but prior genetic studies have been small and have provided conflicting results.[9–12] Accordingly, it remains difficult to identify those at risk, and our lack of biological insights into pathogenesis may help explain why no approved therapies exist to prevent or delay disease progression.

To address the need for large-scale studies of varicose vein disease, we investigated the clinical, environmental, and genetic bases of varicose veins in up to 502 619 individuals from the UK Biobank using a constellation of modalities, including agnostic machine learning; traditional epidemiological analyses; genetic studies, including a genome-wide association study (GWAS) and pathway analyses; and mendelian randomization (MR) to address causality.

METHODS

All data reported in this article are made available to other researchers via application to the UK Biobank for purposes of reproducing the results or replicating the procedure. The data are publicly available at the UK Biobank repository.[13]

Study Design and Participants

We used data from the UK Biobank, a longitudinal cohort study of 502 619 individuals 40 to 69 years of age from 21 centers across the United Kingdom. Between 2006 and 2010, individuals 40 to 69 years of age underwent baseline assessment and have since been carefully investigated for extensive phenotype, lifestyle, health outcomes, and genetic data. The UK Biobank protocol has been described in detail previously[13] and can be found online (https://www.ukbiobank.ac.uk).

In our observational analyses, we excluded individuals with prevalent varicose veins (n=9100), leaving 493 519 individuals eligible. In the GWAS, we studied unrelated individuals of white British ancestry with available phenotype data (n=337 536). The UK Biobank study was approved by the North West Multi-Center Research Ethics Committee, and all participants provided written informed consent to participate in the UK Biobank.

Definitions of Potential Risk Factors

To identify risk factors for varicose veins, we considered all variables from the UK Biobank, spanning domains of demographics, socioeconomic data, lifestyle behaviors, medical history, and a range of physical and cognitive measures. We excluded administrative, temporary, and unspecific *International Classification of Diseases* (*ICD*) codes (chapters U and Z), as well as diagnoses likely to represent varicose veins, including phlebitis, thrombophlebitis, and other diseases of veins and lymphatic vessels (*ICD, 10th Revision* codes I80–I82, I85–I89).

A total of 2715 predictor variables were extracted from the UK Biobank database and used in our phenome-wide scan of varicose vein risk factors (Table I in the online-only Data Supplement). Medical history and smoking status were assessed with self-reported questionnaires. Blood pressure and body mass index (BMI) were measured in a standard fashion (full details available at UK Biobank data showcase; http://biobank.ctsu.ox.ac.uk/crystal/). Systolic blood pressure was defined as the mean of 2 measurements.

Follow-Up and Outcome

The primary outcome was the diagnosis of varicose veins, defined by *ICD, Ninth Revision* (454*) and/or *ICD, 10th Revision* (I-83*). For observational analyses, prevalent cases were excluded (n=9100), leaving 2441 cases of incident varicose veins during a median follow-up of 6.2 years (maximum, 9.1 years). For GWAS and MR analyses, both incident and prevalent events were included (n=11 541). After the exclusion of individuals as a result of genetic relatedness or non-European descent (n=1964), 9577 cases were included.

Machine Learning Analyses

For agnostic discovery of novel risk factors for varicose veins, we used a gradient boosting machine (GBM) model. On the basis of the input predictor variables, this machine learning algorithm consecutively fits new decision trees to provide a more accurate prediction of a response variable (ie, outcome, varicose veins). The principal idea of this algorithm is the learning procedure that results in consecutive error fitting, with each decision tree chosen to minimize the loss function.[14] In addition to classification, GBM ranks the importance (weight) of input variables to the outcome based on decision trees. We used incident cases of varicose veins as the response variable. Results are presented as variable importance. All analyses were conducted with the machine learning platform (H2O 3.10.4.1). Detailed descriptions of the GBM model can be found in Methods in the online-only Data Supplement.

Statistical Analysis

We analyzed the associations of traditional varicose vein risk factors and the top 10 predictors identified through GBM on incident varicose veins using univariable and multivariable Cox regression analyses. For categorical predictors, Kaplan-Meier estimates were used. For continuous predictors, the predictor was entered as a restricted cubic spline to account for any nonlinear associations. The top 10 novel predictors were further assessed with 2 multivariable-adjusted Cox models: adjusting for age and sex and additionally adjusting for established varicose vein risk factors, including BMI, waist-hip ratio (WHR), history of DVT, and pregnancy.[1,2,6–8] As a secondary analysis, we assessed variables in a third model that included the first 5 principal components to account for genetic ancestry. The discriminatory ability of the models was assessed with C indexes. All regression analyses were conducted with R 3.3.0 and 3.3.3.

GWAS of Varicose Veins

A GWAS was performed using imputed genotype data from the second data release of the UK Biobank (9577 cases and 327 959 controls). A detailed description of genotyping, quality control, and imputation can be found in the Methods in the online-only Data Supplement. We included 10 972 371 markers belonging to the Haplotype Reference Consortium 1.1 subset with a minor allele count ≥20 in cases and controls and MaCH r^2>= 0.8. Assuming an additive model, the associations between genotype dosages of each marker and varicose veins were tested in logistic regression (Firth penalized logistic regression in nonconvergence) with PLINK2[15] and adjusted for age, sex, and the first 15 principal components of ancestry. A value of $P<5\times10^{-8}$ was considered to be statistically significant. Independent lead single nucleotide polymorphisms (SNPs) were identified by conditional analysis. Regional plots were created for the association test results of significant loci with Locuszoom version 1.4. All other plots and tables were created in R 3.3.0.

Linkage Disequilibrium Score Regression

Linkage disequilibrium score regression (LDSC) is a tool used to estimate SNP heritability, genetic correlation, and genomic inflation using GWAS summary data sets.[16] Because height appeared as a novel predictor in our analyses, we applied LDSC to estimate the genetic correlation between height and varicose veins using summary statistics from our GWAS for varicose veins and publically available GWAS results for height from the GIANT consortium (Genetic Investigation of Anthropometric Traits).[17] As a comparison, we also examined the genetic correlations with PAD and traditional risk factors for varicose veins: BMI, WHR, and history of DVT. For BMI and WHR, we used GWAS summary data from the GIANT consortium limited to individuals of European ancestry.[18,19] For history of DVT and PAD, GWAS results from the UK Biobank were used.[20] We additionally applied LDSC to estimate SNP heritability and to test for genomic inflation (expected to some degree for polygenic traits and large sample sizes). SNP heritability is reported on the liability scale to take into account sample prevalence of varicose vein disease.

Expression Quantitative Loci Analysis

To explore the potential functional significance of our findings, we evaluated all genome-wide significant loci for evidence of expression quantitative loci (eQTL). Lookups were performed in the Genotype-Tissue Expression database[21] (53 tissue types), and the FUMA GWAS platform was used to interrogate whole-blood data repositories, BIOSQTL, and the blood eQTL browser.[22]

Pathway and Functional Analyses

We used 2 data-driven integrative platforms to perform pathway analyses. After clumping to identify independent loci using PLINK2,[15] we performed analysis using DEPICT.[23] We also used the FUMA GWAS platform to investigate loci for pathway and tissue enrichment.[22] A detailed description of pathway analysis can be found in Methods in the online-only Data Supplement.

Height and Varicose Veins: MR Analysis

We performed a 2-sample MR analysis using height-associated SNPs from the GIANT study, a GWAS meta-analysis of height in 253 288 individuals of European ancestry.[17] A genetic risk score was created with height-associated SNPs as the instrumental variable and our GWAS of varicose veins as the outcome. Our main genetic risk score instrument included 512 SNPs without evidence of pleiotropy (see Methods in the online-only Data Supplement). Analyses were conducted with the R package TwoSampleMR. Power was calculated with the online tool (https://sb452.shinyapps.io/power/).

RESULTS

Machine Learning and Cox Analyses

Baseline characteristics of the study sample are shown in Table 1. The 20 variables with the highest importance in the GBM model are presented in Figure 1. Age, pregnancy, history of DVT, and obesity are previously reported risk factors that were again observed in our study (Table II in the online-only Data Supplement). In addition, several novel predictors of varicose veins were identified, including leg bioimpedance, height, and history of surgery on leg arteries or other major operation. Our GBM model had similar areas under the receiver-operating characteristics curve in the training, validation, and test sets: 0.76, 0.71, and 0.70, with each set having a mean squared error of 0.02, indicating that the model had acceptable discriminatory performance and low error. A number of putative predictors that have been inconsistently reported in the literature, including hypertension (variable 238), age at menopause (variable 405), and smoking (variable 487), were not observed among the top predictors by our machine learning approaches.

The top 10 new predictors were selected, together with previously established risk factors, for additional analysis and validation by 2 Cox models (age and sex adjusted and adjusted for established varicose vein risk factors). Among the independently associated variables were established risk factors such as history of DVT (hazard ratio, 2.60; 95% CI, 2.18–3.11; $P<0.0001$) and BMI (hazard ratio, 1.10; 95% CI 1.03–1.17; $P=0.001$; Table 2) but also novel markers such as leg bioimpedance, greater height, and surgery on leg arteries (Figure 2 and Table 2 and Figure I in the online-only Data Supplement). Each new predictor from our GBM model improved the predictive capacity of the Cox models when added to models including age, sex, and traditional risk factors and remained independently associated in our secondary analysis that also adjusted for genetic ancestry (Table III in the online-only Data Supplement). The largest improvement in the C index was observed for leg bioimpedance and height (from 0.56 to 0.65 and 0.61, respectively; Table IV in the online-only Data Supplement). Of note, debated risk factors such as oral contraceptive use were not found to be independently associated with varicose veins in Cox analysis, whereas some were, including systolic blood pressure.

Table 1. Baseline Characteristics

Characteristic	
Age (n=493 519), y	58 (50–63)
Female sex (n=493 519), % (n)	54 (226 280)
Height (n=491 016), cm	168 (162–175)
Body mass index (n=490 513), kg/m^2	26.8 (24.1–29.9)
Waist-hip ratio (n=491 317)	0.87 (0.80–0.94)
History of deep vein thrombosis (n=491 435), % (n)	2 (9722)
Leg bioimpedance, left (n=483 580), ohms	247 (224–271)
Current smoker (n=490 618), % (n)	11 (52 146)
Duration of moderate daily activity (n=361 856), min	40 (20–60)
Female-specific factors	
Live births (n=272 989), n	1.82 (1.01–2.63)
Age at live first birth (n=180 038), y	25 (22–28)
History of oral contraceptive use (n=263 309), % (n)	3 (6912)
History of hormone replacement therapy (n=263 309), % (n)	7 (19 319)

For continuous variables, medians and interquartile ranges are reported; for categorical variables, percentages and frequencies are given. N is the number of nonmissing values. Moderate daily activity is defined as ≥10 continuous minutes of activities such as carrying light loads or cycling at a normal pace; it does not include walking.

GWAS for Varicose Veins

We conducted a GWAS to identify genetic variants that influence varicose vein susceptibility. Overall, the GWAS results showed modest deviation in test statistics ($\lambda=1.09$). The constructed quantile-quantile plot showed minimal inflation except for in the upper tail of distribution (Figure II in the online-only Data Supplement), suggesting adequate correction for population stratification. LDSC supported this and provided evidence that inflation was caused by a polygenic signal in which heritability accounted for 28% of the population variance in varicose vein disease (LDSC intercept, 1.032, SE=0.0088; $h^2=0.28$, SE=0.024).

We identified 855 new SNPs associated with varicose veins that exceeded genome-wide significance (Figure 3). After conditional analyses, we found 30 independent genetic variants associated with varicose vein disease (Table V in the online-only Data Supplement). The strongest association was located on chromosome 1 in the *CASZ1* gene, in a known blood pressure locus (rs11121615; $P=3.71\times10^{-65}$).[24] Other lead variants were located in the 16q24 region, in or near vascular mechanosensory channel *PIEZO1* and galactosamine-sulfatase enzyme *GALNS* (rs2911461, $P=4.81\times10^{-29}$; rs8053350, $P=8.93\times10^{-18}$). Regional plots for the 30 newly discovered loci are shown in Figure III in the online-only Data Supplement. Full results for all SNPs with values of $P<5\times10^{-8}$ are shown in Table VI in the online-only Data Supplement.

Pathway and Functional Analyses

eQTL analyses identified several genes significantly regulated by varicose vein SNPs. For example, one of the lead variants, rs2861819, had several eQTL associations, including *PNO1, WDR92, PLEK,* and *PPP3R1*, which expresses calcineurin, a critical signaling component during angiogenesis.[25] We also identified significant eQTLs for lead variants rs2911463 (*PIEZO1,*

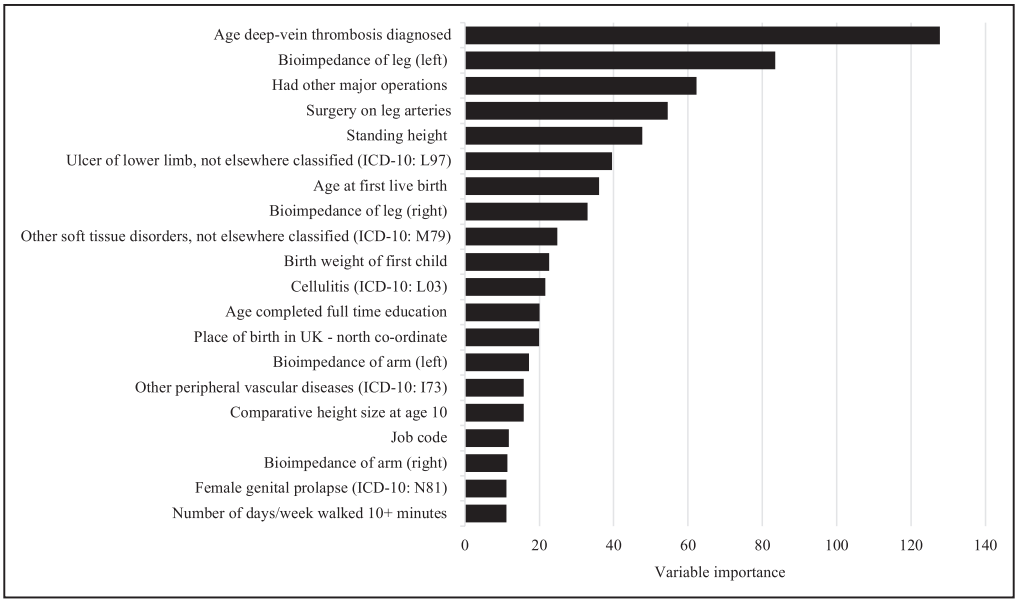

Figure 1. Variable importance for the top 20 variables in the gradient boosting model for varicose veins.
ICD-10 indicates *International Classification of Diseases, 10th revision.*

P=1.50×10^{-5}; *GALNS*, P=1.50×10^{-5}) and rs3101725 (*FBN2*, P=9.50×10^{-7}). These loci are particularly interesting because mutations in *GALNS* and *FBN2* have been linked to a hereditary form of skeletal dysplasia[26] and the Marfan-like syndrome congenital contractural arachnodactyly,[27] respectively. Detailed results from eQTL analysis are given in Table VII in the online-only Data Supplement.

FUMA prioritized tissues, pathways, and gene sets enriched in varicose vein loci. Tissue enrichment was greatest for adipose tissue (P=7.58×10^{-5}; Table VIII in the online-only Data Supplement). Evaluation for enriched biological processes identified several gene sets related to vascular development and structure (vasculature development, P=1.96×10^{-6}; endothelial cell differentiation, P=8.95×10^{-6}) and highlighted canonical pathways that may influence varicose vein disease: Gata3 pathway (P=3.06×10^{-6}), vascular endothelial growth factor signaling (P=1.11×10^{-5}), and nuclear factor of activated T cells pathway (P=1.29×10^{-4}; Table IX in the online-only Data Supplement).

In addition, FUMA analysis revealed that our lead SNPs were previously associated with a variety of traits, including varicose vein predictors identified in our observational analyses: WHR (P=2.79×10^{-4}) and height (P=2.30×10^{-3}). No significantly enriched gene sets were identified by DEPICT that met the false discovery rate cutoff of <0.05.

Genetic Correlation Between Height and Varicose Veins

After integrating data from our GWAS and publicly available GWAS summary results on height, we found evidence for a strong genetic correlation between height and varicose veins (r_g=0.16; SE=0.031; P=2.99×10^{-7}). This suggests that there is ≈16% genetic overlap between height and varicose veins, which was larger than the estimated genetic correlation with BMI (r_g=0.095; SE=0.032; P=0.00260) and WHR (r_g=0.078; SE=0.052; P=0.131). History of DVT was found to have the greatest genetic correlation with varicose vein disease (r_g=0.36; SE=0.0805; P=5.57×10^{-7}) compared with PAD (r_g=0.20; SE=0.22; P=0.37) and traditional factors such as BMI. The estimated genetic correlation of varicose veins and PAD was lower than that of DVT and was nonsignificant.

Mendelian Randomization

Because we observed a strong association of height with varicose veins, we next performed multiple MR analyses to investigate causal effects. We used a genetic risk score instrument composed of 512 independent SNPs previously associated with height,[17] without evidence of pleiotropy. The results provided strong evidence of a causal effect of height on varicose veins (inverse-variance weighted: odds ratio [OR], 1.26, P=2.07×10^{-16}; weighted median: OR, 1.28, P=1.79×10^{-8}; maximum likelihood: OR, 1.26, P=9.12×10^{-18}; MR Egger: OR, 1.43, P=6.74×10^{-6}; Table X in the online-only Data Supplement). These results remained consistent in a leave-one-out sensitivity analysis, and a funnel plot did not indicate any directional pleiotropy (Figures IV and V in the online-only Data Supplement). Sensitivity analyses including all 536 variants (without exclusion of potentially pleiotropic variants) provided almost identical results

Table 2. Cox Models of The Top Novel Predictors From the Gradient Boosting Machine approach, Along With Debated Predictors for Varicose Veins

Variable		Age- and Sex-Adjusted Hazard Ratio (95% CI)	Fully Adjusted* Hazard Ratio (95% CI)
Traditional	History of deep vein thrombosis	2.71 (2.28–3.23)	2.60 (2.18–3.11)
	Birth weight of first child†	1.23 (1.05–1.44)	1.21 (1.03–1.42)
	Number of live births	1.12 (0.99–1.27)	1.12 (0.99–1.27)
	Body mass index	1.11 (1.00–1.24)	1.11 (0.99–1.24)
	Waist-hip ratio	1.10 (0.97–1.26)	1.01 (0.88–1.16)
Novel	Leg bioimpedance	0.56 (0.51–0.63)	0.43 (0.39–0.50)
	Height	1.70 (1.48–1.96)	1.74 (1.51–2.01)
	Major operations	0.75 (0.69–0.82)	0.77 (0.70–0.84)
	Surgery on leg arteries	3.71 (2.46–5.62)	3.50 (2.30–5.33)
	Ulceration of lower limb (*ICD-10* code L97)	4.65 (2.36–9.16)	3.00 (1.36–6.61)
	Other soft tissue disorders (*ICD-10* code M79)	2.01 (1.58–2.55)	1.66 (1.30–2.12)
	Cellulitis (*ICD-10* code L03)	2.09 (1.52–2.87)	1.79 (1.29–2.48)
Debated	Smoking	1.04 (0.92–1.19)	1.04 (0.91–1.19)
	Systolic blood pressure	0.81 (0.72–0.90)	0.79 (0.71–0.88)
	Age completed full-time education	0.80 (0.70–0.91)	0.82 (0.72–0.93)
	Oral contraceptive use	0.67 (0.44–1.02)	0.68 (0.44–1.04)
	Hormone replacement therapy	1.25 (1.04–1.49)	1.25 (1.04–1.50)
	Days per week walked >10 min	1.32 (1.18–1.47)	1.33 (1.19–1.48)
	Duration of moderate daily activity	1.00 (0.87–1.14)	1.00 (0.88–1.15)

Associations between candidate variables and varicose veins are reported as hazard ratio and 95% CI for the upper versus lower quartile for continuous variables or presence versus absence for binary variables. Separate models were used for each variable.

ICD-10 indicates *International Classification of Diseases, 10th Revision*.

*Fully adjusted model includes age, sex, body mass index, history of deep vein thrombosis, and waist-hip ratio. Pregnancy-specific factors, oral contraceptive use, and hormone replacement therapy were assessed only in female study participants.

†Weight in pounds.

but with a larger degree of heterogeneity, as expected (Table X in the online-only Data Supplement). At an α threshold of 0.05, power for MR analyses was estimated to be 100% with an OR of 1.51 per 1-SD increase in height (corresponding to the effect from our observational analyses).

DISCUSSION

Leveraging data from 493 519 individuals, this study is the largest to investigate the genetic and environmental bases of varicose veins. First, we used "hypothesis-free" machine learning approaches to identify clinical and environmental predictors of disease. This powerful method confirmed a number of well-established relationships and identified a variety of novel risk factors that have not been previously reported. Second, we used multivariable Cox regression methods to validate our epidemiological observations and confirm their importance for predicting disease risk. Third, we performed a GWAS and pathway analyses to discover genetic determinants of varicose veins. Finally, we used MR to establish height as a causal risk factor for disease.

Environmental Predictors of Varicose Veins

Prior studies have yielded inconsistencies in environmental predictors of varicose veins. Machine learning confirmed several of the more accepted risk factors (eg, age, history of DVT, obesity, pregnancy),[1,2,6–8] in addition to providing insight into previously debated predictors. Age when full-time education was completed (reflecting length of education) was among the most important variables identified in the GBM model, which supports the association between education and varicose veins that has been observed in smaller studies.[8,28] It is possible that this association is related to the ability to obtain medical treatment because level of education may reflect socioeconomic circumstances and access to health care. Individuals with higher education may be more inclined to seek and receive medical care for varicose veins and thus receive a formal diagnosis. Previous studies have also found a significant association with hormone replacement therapy,[2,28] which again appeared as an important variable in our GBM model and was positively related to incident varicose veins in multivariable analysis. Supplemental estrogen may alter venous wall compliance; a previous study showed that elevated levels of endogenous estradiol are associated with increased venous distensibility.[29]

On the contrary, machine learning did not prioritize several other controversial factors (eg, oral contraceptive use; Table 2),[2,6,8,28] which remained consistent in subsequent formal Cox regression analyses (investigating 1 risk factor at a time). Given the size of this study, it is likely that these previously debated factors have limited utility as traditional risk discriminators.

Physical activity, smoking, and hypertension are other debated risk factors for varicose vein disease. Although duration of moderate daily activity was not found to be a strong predictor in our GBM model and

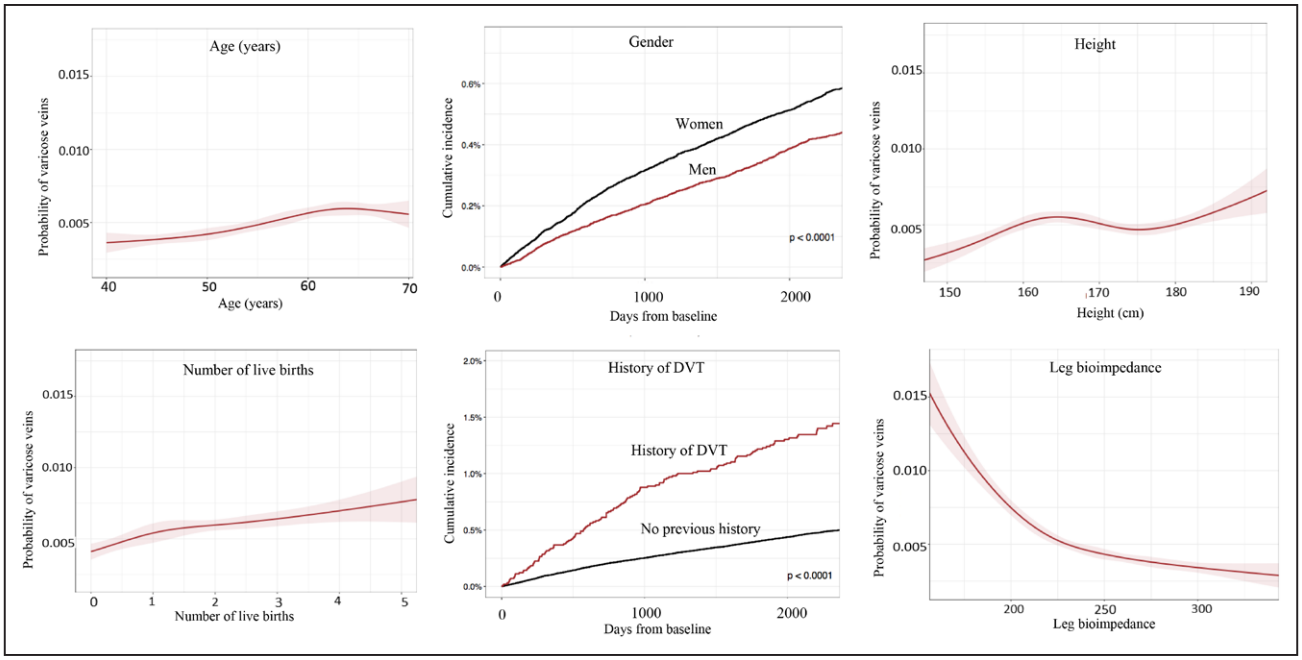

Figure 2. Univariable analysis demonstrating relationships between varicose veins and other variables.
DVT indicates deep vein thrombosis.

Cox analyses, the number of days per weeks of walking ≥10 minutes was positively associated. This difference may reflect a strong influence of immobility and nonambulatory status on disease rather than routine aerobic exercise per se. Some studies have reported higher smoking rates in subjects with varicose veins compared with those without disease.[6,30] However, the majority of studies have observed no association between varicose veins and smoking.[31–35] In our study, smoking was not associated with varicose vein development after adjustment for traditional risk factors but became marginally associated in our secondary analysis that adjusted for genetic ancestry. Studies of the association between varicose veins and hypertension have also shown various relationships, including a positive association,[6] no association,[7] and a lower prevalence of hypertension among subjects with varicose veins.[8] In multivariable analysis, we observed a slight

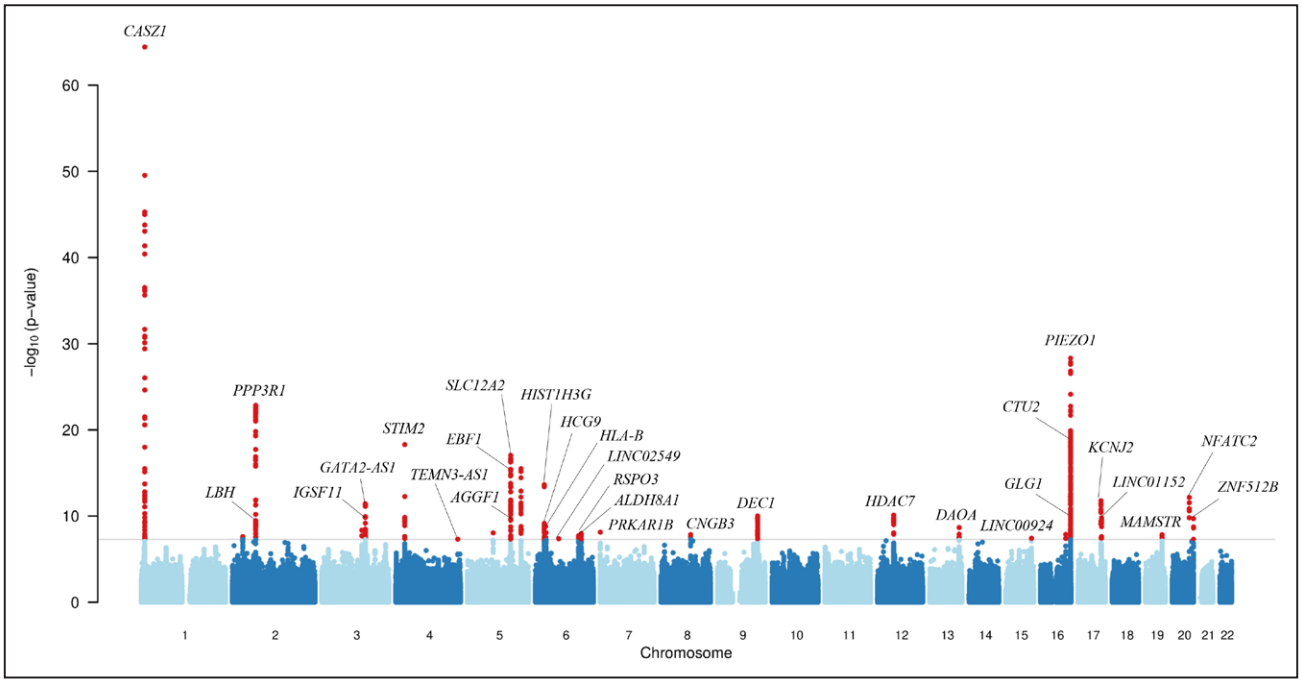

Figure 3. Manhattan plot of genome-wide association study analysis.
Gray line represents the threshold of genome-wide significance, $P<5\times10^{-8}$. Gene names correspond to the gene in closest proximity to the variant.

inverse association between systolic blood pressure and varicose veins. Given this modest association of varicose veins with both smoking and blood pressure, which could reflect reverse causation or confounding, more information is needed to evaluate their potential shared pathophysiology with varicose veins.

Because our machine learning algorithms are not restricted to a priori hypotheses, they had the capacity to identify novel factors associated with disease. By using this "agnostic" phenome-wide approach, we were able to identify several new strong predictors, including leg bioimpedance and height. Height was suggested to be a potential risk factor in an early epidemiological study several decades ago[31] but has been inconsistently reported since then.[7,28,31] Bioimpedance, defined as the ability of tissue to impede electric current, is related to the amount of fluid accumulation in body tissue.[36] Combined with the recent finding that leg bioimpedance is associated with risk for heart failure,[37] these novel predictors suggest a link between high-volume venous reflux, increased hydrostatic pressure, and resulting venous hypertension.

Genetics of Varicose Veins

Although varicose veins are known to have a strong heritable component,[9] their pathophysiological underpinnings remain poorly understood. To date, genetic research on varicose veins has been restricted to syndromic forms of disease (eg, Klippel-Trenaunay syndrome) and candidate gene studies in patients with primary venous disease.[38] These studies have suggested that mutations in *FOXC2*, thrombomodulin (*THBD*), and desmuslin (*SYNM*) may promote the development of varicose veins by altering vein function. However, these studies were typically small (18–700 patients), and few confirmation studies have been reported.

In our GWAS, we included nearly 10 000 cases and 300 000 controls; thus, our analyses were uniquely powered to screen the whole genome in an unbiased manner. We identified 30 genetic loci robustly associated with varicose veins, with the strongest associations occurring in the intron region of *CASZ1* (rs11121615; $P=3.71\times10^{-65}$), which has been implicated in blood pressure,[24] and in 16q24, where, among other genes, *PIEZO1,* encoding a vascular mechanosensory channel, is located (rs2911463; $P=4.81\times10^{-29}$). Disruption of *PIEZO1* has previously been shown to result in significant disorganization of the vascular system, suggesting the importance of *PIEZO1* for mature vascular development.[39] In addition, the lead SNP rs7773004 on 6q14 is located within 50 kB upstream of the hemochromatosis gene *HFE*, in which mutations have previously been associated with both venous ulceration and venous thromboembolism (an aggregate of DVT and pulmonary embolism).[40,41] These loci appear particularly interesting given the strong genetic overlap that we discovered between varicose veins and DVT. Genes regulating vascular dysfunction may promote changes in venous architecture and chronic blood stasis (and could represent new translational targets).

In a comparison of the genetic correlation of varicose veins with traditional risk factors and PAD, LDSC demonstrated the strongest genetic correlation with history of DVT. Several genes have previously been linked to both varicose veins and DVT, including *THBD*[42] and methylenetetrahydrofolate reductase (*MTHFR*).[43] Mutations in these genes are classically associated with inherited hypercoagulability, but patients with varicose veins have also been found to have a higher prevalence of thrombophilias and increased levels of systemic inflammatory and prothrombotic markers.[44–46] In addition, a nationwide genetic study in Sweden demonstrated that varicose veins and venous thromboembolism share familial susceptibility.[47] These findings similarly support the shared genetic associations between varicose veins and DVT and suggest an overlap in their underlying pathophysiology.

To gain further biological insights from our GWAS, we performed a series of analyses to identify genes and pathways that may be dysregulated in individuals predisposed to varicose vein formation (Tables VII through IX in the online-only Data Supplement). eQTL analyses suggested that several SNPs are associated with altered in vivo expression of genes that have been related to vascular development/integrity (eg, *PPP3R1*[25], *PIEZO1,*[39] *SOX18*[48]), limb development (eg, *LBH*[49]), and conditions associated with skeletal abnormalities (eg, *GALNS,*[26] *FBN2*[27]) (Table VII in the online-only Data Supplement). The potential importance of genes related to vascular development ($P=1.96\times10^{-6}$), limb development ($P=3.55\times10^{-3}$), and height ($P=2.30\times10^{-3}$) was also highlighted in pathway analyses (Table IX in the online-only Data Supplement). In particular, genetic variants associated with varicose veins were found to be enriched for the vascular endothelial growth factor pathway and nuclear factor of activated T cells pathway. Vascular endothelial growth factor signaling is required for vessel formation and homeostasis, whereas the nuclear factor of activated T cells pathway regulates intracellular calcium signaling in osteoblasts and osteoclasts during bone growth and remodeling.[50] These findings warrant future studies to experimentally evaluate the reported genetic loci.

Because height was associated with varicose veins in our observational analyses, we concluded our studies with LDSC and MR analyses that demonstrated a shared genetic pathogenesis and confirmed a strong causal relationship between increased height and varicose veins (Figures IV and V and Table X in the online-only Data Supplement). Height has also been found to be an independent predictor for venous thromboem-

bolism.[51] Considering this, our findings suggest that height may have consequences on long-term risk for both varicose veins and the associated complications.

Study Strengths and Limitations

Strengths of our study include the large sample size and use of combined epidemiological and genetic studies, making it the largest and most comprehensive study of varicose veins to date. Nevertheless, this study has important limitations. The UK Biobank includes predominantly white middle-aged to elderly individuals, so our conclusions may have limited generalizability to people of other ethnicities and age groups (such as younger people who have not yet developed disease). Our epidemiological analysis identified incident events with hospital *ICD* codes. Although this allowed standardized identification of varicose vein events, the true incidence of varicose veins may also be underrepresented in this data set because diagnosis required a formal *ICD* code input and does not capture data from individuals who did not seek medical attention or include cases of self-reported varicose veins. Thus, it is possible that the controls in our analysis included undocumented cases of varicose veins. This may introduce bias and underestimate some of the associations, driving them toward the null. It is thought that the potential bias could lead to false-positive associations. In addition, limitations in characterizing candidate variables such as physical activity, job, and diet may have altered the strength of associations in our observational analyses, which could be a reason why some of the debated risk factors did not show significant associations. Lastly, our analysis does not include the relation of our findings to disease management. The goal of this study was to identify environmental and genetic risk factors for varicose veins. Thus, although our discoveries may help predict or treat varicose veins in the future, the study did not focus on their impact on current patient care.

Conclusions

Using a large study to investigate varicose vein disease, our study substantially extends our understanding of the genetic and environmental bases of varicose veins. We demonstrate that greater height is associated with greater susceptibility to varicose vein disease. Several risk factors with importance for predicting disease risk were identified, including previously debated (hormone replacement therapy, length of education) and other novel (leg bioimpedance, history of cellulitis, surgery on leg arteries) predictors. Our discovery of genetic determinants of disease also sets a path for identification of new targets in the effort to develop therapy for varicose veins.

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REFERENCES

1. Hamdan A. Management of varicose veins and venous insufficiency. *JAMA*. 2012;308:2612–2621. doi: 10.1001/jama.2012.111352
2. Beebe-Dimmer JL, Pfeifer JR, Engle JS, Schottenfeld D. The epidemiology of chronic venous insufficiency and varicose veins. *Ann Epidemiol*. 2005;15:175–184. doi: 10.1016/j.annepidem.2004.05.015
3. Chang SL, Huang YL, Lee MC, Hu S, Hsiao YC, Chang SW, Chang CJ, Chen PC. Association of varicose veins with incident venous thromboembolism and peripheral artery disease. *JAMA*. 2018;319:807–817. doi: 10.1001/jama.2018.0246
4. Müller-Bühl U, Leutgeb R, Engeser P, Achankeng EN, Szecsenyi J, Laux G. Varicose veins are a risk factor for deep venous thrombosis in general practice patients. *Vasa*. 2012;41:360–365. doi: 10.1024/0301-1526/a000222
5. Mäkivaara LA, Ahti TM, Luukkaala T, Hakama M, Laurikka JO. Persons with varicose veins have a high subsequent incidence of arterial disease: a population-based study in Tampere, Finland. *Angiology*. 2007;58:704–709. doi: 10.1177/0003319707299202
6. Brand FN, Dannenberg AL, Abbott RD, Kannel WB. The epidemiology of varicose veins: the Framingham study. *Am J Prev Med*. 1988;4:96–101.
7. Sisto T, Reunanen A, Laurikka J, Impivaara O, Heliövaara M, Knekt P, Aromaa A. Prevalence and risk factors of varicose veins in lower extremities: Mini-Finland Health Survey. *Eur J Surg*. 1995;161:405–414.

8. Scott TE, LaMorte WW, Gorin DR, Menzoian JO. Risk factors for chronic venous insufficiency: a dual case-control study. *J Vasc Surg*. 1995;22: 622–628.
9. Brinsuk M, Tank J, Luft FC, Busjahn A, Jordan J. Heritability of venous function in humans. *Arterioscler Thromb Vasc Biol*. 2004;24:207–211. doi: 10.1161/01.ATV.0000107080.48969.64
10. Ahti TM, Mäkivaara LA, Luukkaala T, Hakama M, Laurikka JO. Effect of family history on the risk of varicose veins is affected by differential misclassification. *J Clin Epidemiol*. 2010;63:686–690. doi: 10.1016/j.jclinepi.2009.10.003
11. Cornu-Thenard A, Boivin P, Baud JM, De Vincenzi I, Carpentier PH. Importance of the familial factor in varicose disease: clinical study of 134 families. *J Dermatol Surg Oncol*. 1994;20:318–326.
12. Krysa J, Jones GT, van Rij AM. Evidence for a genetic role in varicose veins and chronic venous insufficiency. *Phlebology*. 2012;27:329–335. doi: 10.1258/phleb.2011.011030
13. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779
14. Natekin A, Knoll A. Gradient boosting machines, a tutorial. *Front Neurorobot*. 2013;7:21. doi: 10.3389/fnbot.2013.00021
15. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4:7. doi: 10.1186/s13742-015-0047-8
16. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Patterson N, Daly MJ, Price AL, Neale BM; Schizophrenia Working Group of the Psychiatric Genomics Consortium. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015;47:291–295. doi: 10.1038/ng.3211
17. Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, Chu AY, Estrada K, Luan J, Kutalik Z, Amin N, Buchkovich ML, Croteau-Chonka DC, Day FR, Duan Y, Fall T, Fehrmann R, Ferreira T, Jackson AU, Karjalainen J, Lo KS, Locke AE, Mägi R, Mihailov E, Porcu E, Randall JC, Scherag A, Vinkhuyzen AA, Westra HJ, Winkler TW, Workalemahu T, Zhao JH, Absher D, Albrecht E, Anderson D, Baron J, Beekman M, Demirkan A, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Fraser RM, Goel A, Gong J, Justice AE, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Lui JC, Mangino M, Mateo Leach I, Medina-Gomez C, Nalls MA, Nyholt DR, Palmer CD, Pasko D, Pechlivanis S, Prokopenko I, Ried JS, Ripke S, Shungin D, Stancáková A, Strawbridge RJ, Sung YJ, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Afzal U, Arnlöv J, Arscott GM, Bandinelli S, Barrett A, Bellis C, Bennett AJ, Berne C, Blüher M, Bolton JL, Böttcher Y, Boyd HA, Bruinenberg M, Buckley BM, Buyske S, Caspersen IH, Chines PS, Clarke R, Claudi-Boehm S, Cooper M, Daw EW, De Jong PA, Deelen J, Delgado G, Denny JC, Dhonukshe-Rutten R, Dimitriou M, Doney AS, Dörr M, Eklund N, Eury E, Folkersen L, Garcia ME, Geller F, Giedraitis V, Go AS, Grallert H, Grammer TB, Gräßler J, Grönberg H, de Groot LC, Groves CJ, Haessler J, Hall P, Haller T, Hallmans G, Hannemann A, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hemani G, Henders AK, Hillege HL, Hlatky MA, Hoffmann W, Hoffmann P, Holmen O, Houwing-Duistermaat JJ, Illig T, Isaacs A, James AL, Jeff J, Johansen B, Johansson Å, Jolley J, Juliusdottir T, Junttila J, Kho AN, Kinnunen L, Klopp N, Kocher T, Kratzer W, Lichtner P, Lind L, Lindström J, Lobbens S, Lorentzon M, Lu Y, Lyssenko V, Magnusson PK, Mahajan A, Maillard M, McArdle WL, McKenzie CA, McLachlan S, McLaren PJ, Menni C, Merger S, Milani L, Moayyeri A, Monda KL, Morken MA, Müller G, Müller-Nurasyid M, Musk AW, Narisu N, Nauck M, Nolte IM, Nöthen MM, Oozageer L, Pilz S, Rayner NW, Renstrom F, Robertson NR, Rose LM, Roussel R, Sanna S, Scharnagl H, Scholtens S, Schumacher FR, Schunkert H, Scott RA, Sehmi J, Seufferlein T, Shi J, Silventoinen K, Smit JH, Smith AV, Smolonska J, Stanton AV, Stirrups K, Stott DJ, Stringham HM, Sundström J, Swertz MA, Syvänen AC, Tayo BO, Thorleifsson G, Tyrer JP, van Dijk S, van Schoor NM, van der Velde N, van Heemst D, van Oort FV, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Waldenberger M, Wennauer R, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, Arveiler D, Bakker SJ, Beilby J, Bergman RN, Bergmann S, Biffar R, Blangero J, Boomsma DI, Bornstein SR, Bovet P, Brambilla P, Brown MJ, Campbell H, Caulfield MJ, Chakravarti A, Collins R, Collins FS, Crawford DC, Cupples LA, Danesh J, de Faire U, den Ruijter HM, Erbel R, Erdmann J, Eriksson JG, Farrall M, Ferrannini E, Ferrières J, Ford I, Forouhi NG, Forrester T, Gansevoort RT, Gejman PV, Gieger C, Golay A, Gottesman O, Gudnason V, Gyllensten U, Haas DW, Hall AS, Harris TB, Hattersley AT, Heath AC, Hengstenberg C, Hicks AA, Hindorff LA, Hingorani AD, Hofman A, Hovingh GK, Humphries SE, Hunt SC, Hypponen E, Jacobs KB, Jarvelin MR, Jousilahti P, Jula AM, Kaprio J, Kastelein JJ, Kayser M, Kee F, Keinanen-Kiukaanniemi SM, Kiemeney LA, Kooner JS, Kooperberg C, Koskinen S, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Le Marchand L, Lehtimäki T, Lupoli S, Madden PA, Männistö S, Manunta P, Marette A, Matise TC, McKnight B, Meitinger T, Moll FL, Montgomery GW, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Ouwehand WH, Pasterkamp G, Peters A, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ritchie M, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schwarz PE, Sebert S, Sever P, Shuldiner AR, Sinisalo J, Steinthorsdottir V, Stolk RP, Tardif JC, Tönjes A, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PI, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hayes MG, Hui J, Hunter DJ, Hveem K, Jukema JW, Kaplan RC, Kivimaki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CN, Pedersen NL, Perola M, Pérusse L, Peters U, Powell JE, Power C, Quertermous T, Rauramaa R, Reinmaa E, Ridker PM, Rivadeneira F, Rotter JI, Saaristo TE, Saleheen D, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Strauch K, Stumvoll M, Tuomilehto J, Uusitupa M, van der Harst P, Völzke H, Walker M, Wareham NJ, Watkins H, Wichmann HE, Wilson JF, Zanen P, Deloukas P, Heid IM, Lindgren CM, Mohlke KL, Speliotes EK, Thorsteinsdottir U, Barroso I, Fox CS, North KE, Strachan DP, Beckmann JS, Berndt SI, Boehnke M, Borecki IB, McCarthy MI, Metspalu A, Stefansson K, Uitterlinden AG, van Duijn CM, Franke L, Willer CJ, Price AL, Lettre G, Loos RJ, Weedon MN, Ingelsson E, O'Connell JR, Abecasis GR, Chasman DI, Goddard ME, Visscher PM, Hirschhorn JN, Frayling TM; Electronic Medical Records and Genomics (eMEMERGEGE) Consortium; MIGen Consortium; PAGEGE Consortium; LifeLines Cohort Study. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet*. 2014;46:1173–1186. doi: 10.1038/ng.3097
18. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC, Esko T, Fall T, Ferreira T, Gustafsson S, Kutalik Z, Luan J, Mägi R, Randall JC, Winkler TW, Wood AR, Workalemahu T, Faul JD, Smith JA, Zhao JH, Zhao W, Chen J, Fehrmann R, Hedman ÅK, Karjalainen J, Schmidt EM, Absher D, Amin N, Anderson D, Beekman M, Bolton JL, Bragg-Gresham JL, Buyske S, Demirkan A, Deng G, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Goel A, Gong J, Jackson AU, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Mangino M, Leach IM, Medina-Gomez C, Medland SE, Nalls MA, Palmer CD, Pasko D, Pechlivanis S, Peters MJ, Prokopenko I, Shungin D, Stančáková A, Strawbridge RJ, Sung YJ, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Isaacs A, Albrecht E, Ärnlöv J, Arscott GM, Attwood AP, Bandinelli S, Barrett A, Bas IN, Bellis C, Bennett AJ, Berne C, Blagieva R, Blüher M, Böhringer S, Bonnycastle LL, Böttcher Y, Boyd HA, Bruinenberg M, Caspersen IH, Chen YI, Clarke R, Daw EW, de Craen AJM, Delgado G, Dimitriou M, Doney ASF, Eklund N, Estrada K, Eury E, Folkersen L, Fraser RM, Garcia ME, Geller F, Giedraitis V, Gigante B, Go AS, Golay A, Goodall AH, Gordon SD, Gorski M, Grabe HJ, Grallert H, Grammer TB, Gräßler J, Grönberg H, Groves CJ, Gusto G, Haessler J, Hall P, Haller T, Hallmans G, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hengstenberg C, Holmen O, Hottenga JJ, James AL, Jeff JM, Johansson Å, Jolley J, Juliusdottir T, Kinnunen L, Koenig W, Koskenvuo M, Kratzer W, Laitinen J, Lamina C, Leander K, Lee NR, Lichtner P, Lind L, Lindström J, Lo KS, Lobbens S, Lorbeer R, Lu Y, Mach F, Magnusson PKE, Mahajan A, McArdle WL, McLachlan S, Menni C, Merger S, Mihailov E, Milani L, Moayyeri A, Monda KL, Morken MA, Mulas A, Müller G, Müller-Nurasyid M, Musk AW, Nagaraja R, Nöthen MM, Nolte IM, Pilz S, Rayner NW, Renstrom F, Rettig R, Ried JS, Ripke S, Robertson NR, Rose LM, Sanna S, Scharnagl H, Scholtens S, Schumacher FR, Scott WR, Seufferlein T, Shi J, Smith AV, Smolonska J, Stanton AV, Steinthorsdottir V, Stirrups K, Stringham HM, Sundström J, Swertz MA, Swift AJ, Syvänen AC, Tan ST, Tayo BO, Thorand B, Thorleifsson G, Tyrer JP, Uh HW, Vandenput L, Verhulst FC, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Warren HR, Waterworth D, Weedon MN, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, Brennan EP, Choi M, Dastani Z, Drong AW, Eriksson P, Franco-Cereceda A, Gådin JR, Gharavi AG, Goddard ME, Handsaker RE, Huang J, Karpe F, Kathiresan S, Keildson S, Kiryluk K, Kubo M, Lee JY, Liang L, Lifton RP, Ma B, McCarroll SA, McKnight AJ, Min JL, Moffatt MF, Montgomery GW,

Murabito JM, Nicholson G, Nyholt DR, Okada Y, Perry JRB, Dorajoo R, Reinmaa E, Salem RM, Sandholm N, Scott RA, Stolk L, Takahashi A, Tanaka T, van 't Hooft FM, Vinkhuyzen AAE, Westra HJ, Zheng W, Zondervan KT, Heath AC, Arveiler D, Bakker SJL, Beilby J, Bergman RN, Blangero J, Bovet P, Campbell H, Caulfield MJ, Cesana G, Chakravarti A, Chasman DI, Chines PS, Collins FS, Crawford DC, Cupples LA, Cusi D, Danesh J, de Faire U, den Ruijter HM, Dominiczak AF, Erbel R, Erdmann J, Eriksson JG, Farrall M, Felix SB, Ferrannini E, Ferrières J, Ford I, Forouhi NG, Forrester T, Franco OH, Gansevoort RT, Gejman PV, Gieger C, Gottesman O, Gudnason V, Gyllensten U, Hall AS, Harris TB, Hattersley AT, Hicks AA, Hindorff LA, Hingorani AD, Hofman A, Homuth G, Hovingh GK, Humphries SE, Hunt SC, Hyppönen E, Illig T, Jacobs KB, Jarvelin MR, Jöckel KH, Johansen B, Jousilahti P, Jukema JW, Jula AM, Kaprio J, Kastelein JJP, Keinanen-Kiukaanniemi SM, Kiemeney LA, Knekt P, Kooner JS, Kooperberg C, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Marchand LL, Lehtimäki T, Lyssenko V, Männistö S, Marette A, Matise TC, McKenzie CA, McKnight B, Moll FL, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Madden PAF, Pasterkamp G, Peden JF, Peters A, Postma DS, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ridker PM, Rioux JD, Ritchie MD, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schunkert H, Schwarz PEH, Sever P, Shuldiner AR, Sinisalo J, Stolk RP, Strauch K, Tönjes A, Trégouët DA, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Völker U, Waeber G, Willemsen G, Witteman JC, Zillikens MC, Adair LS, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bornstein SR, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PIW, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hui J, Hunter DJ, Hveem K, Kaplan RC, Kivimaki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Metspalu A, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CNA, Pedersen NL, Perola M, Pérusse L, Peters U, Power C, Quertermous T, Rauramaa R, Rivadeneira F, Saaristo TE, Saleheen D, Sattar N, Schadt EE, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Thorsteinsdottir U, Stumvoll M, Tuomilehto J, Uitterlinden AG, Uusitupa M, van der Harst P, Walker M, Wallaschofski H, Wareham NJ, Watkins H, Weir DR, Wichmann HE, Wilson JF, Zanen P, Borecki IB, Deloukas P, Fox CS, Heid IM, O'Connell JR, Strachan DP, Stefansson K, van Duijn CM, Abecasis GR, Franke L, Frayling TM, McCarthy MI, Visscher PM, Scherag A, Willer CJ, Boehnke M, Mohlke KL, Lindgren CM, Beckmann JS, Barroso I, North KE, Ingelsson E, Hirschhorn JN, Loos RJF, Speliotes EK; LifeLines Cohort Study; ADIPOGen Consortium; AGEN-BMI Working Group; CARDIOGRAMplusC4D Consortium; CKDGen Consortium; GLGC; ICBP; MAGIC Investigators; MuTHER Consortium; MIGen Consortium; PAGE Consortium; ReproGen Consortium; GENIE Consortium; International Endogene Consortium. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518:197–206. doi: 10.1038/nature14177

19. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, Thorleifsson G, Zillikens MC, Speliotes EK, Mägi R, Workalemahu T, White CC, Bouatia-Naji N, Harris TB, Berndt SI, Ingelsson E, Willer CJ, Weedon MN, Luan J, Vedantam S, Esko T, Kilpeläinen TO, Kutalik Z, Li S, Monda KL, Dixon AL, Holmes CC, Kaplan LM, Liang L, Min JL, Moffatt MF, Molony C, Nicholson G, Schadt EE, Zondervan KT, Feitosa MF, Ferreira T, Lango Allen H, Weyant RJ, Wheeler E, Wood AR, Estrada K, Goddard ME, Lettre G, Mangino M, Nyholt DR, Purcell S, Smith AV, Visscher PM, Yang J, McCarroll SA, Nemesh J, Voight BF, Absher D, Amin N, Aspelund T, Coin L, Glazer NL, Hayward C, Heard-Costa NL, Hottenga JJ, Johansson A, Johnson T, Kaakinen M, Kapur K, Ketkar S, Knowles JW, Kraft P, Kraja AT, Lamina C, Leitzmann MF, McKnight B, Morris AP, Ong KK, Perry JR, Peters MJ, Polasek O, Prokopenko I, Rayner NW, Ripatti S, Rivadeneira F, Robertson NR, Sanna S, Sovio U, Surakka I, Teumer A, van Wingerden S, Vitart V, Zhao JH, Cavalcanti-Proença C, Chines PS, Fisher E, Kulzer JR, Lecoeur C, Narisu N, Sandholt C, Scott LJ, Silander K, Stark K, Tammesoo ML, Teslovich TM, Timpson NJ, Watanabe RM, Welch R, Chasman DI, Cooper MN, Jansson JO, Kettunen J, Lawrence RW, Pellikka N, Perola M, Vandenput L, Alavere H, Almgren P, Atwood LD, Bennett AJ, Biffar R, Bonnycastle LL, Bornstein SR, Buchanan TA, Campbell H, Day IN, Dei M, Dörr M, Elliott P, Erdos MR, Eriksson JG, Freimer NB, Fu M, Gaget S, Geus EJ, Gjesing AP, Grallert H, Grässler J, Groves CJ, Guiducci C, Hartikainen AL, Hassanali N, Havulinna AS, Herzig KH, Hicks AA, Hui J, Igl W, Jousilahti P, Jula A, Kajantie E, Kinnunen L, Kolcic I, Koskinen S, Kovacs P, Kroemer HK, Krzelj V, Kuusisto J, Kvaloy K, Laitinen J, Lantieri O, Lathrop GM, Lokki ML, Luben RN, Ludwig B, McArdle WL, McCarthy A, Morken MA, Nelis M, Neville MJ, Paré G, Parker AN, Peden JF, Pichler I, Pietiläinen KH, Platou CG, Pouta A, Ridderstråle M, Samani NJ, Saramies J, Sinisalo J, Smit JH, Strawbridge RJ, Stringham HM, Swift AJ, Teder-Laving M, Thomson B, Usala G, van Meurs JB, van Ommen GJ, Vatin V, Volpato CB, Wallaschofski H, Walters GB, Widen E, Wild SH, Willemsen G, Witte DR, Zgaga L, Zitting P, Beilby JP, James AL, Kähönen M, Lehtimäki T, Nieminen MS, Ohlsson C, Palmer LJ, Raitakari O, Ridker PM, Stumvoll M, Tönjes A, Viikari J, Balkau B, Ben-Shlomo Y, Bergman RN, Boeing H, Smith GD, Ebrahim S, Froguel P, Hansen T, Hengstenberg C, Hveem K, Isomaa B, Jørgensen T, Karpe F, Khaw KT, Laakso M, Lawlor DA, Marre M, Meitinger T, Metspalu A, Midthjell K, Pedersen O, Salomaa V, Schwarz PE, Tuomi T, Tuomilehto J, Valle TT, Wareham NJ, Arnold AM, Beckmann JS, Bergmann S, Boerwinkle E, Boomsma DI, Caulfield MJ, Collins FS, Eiriksdottir G, Gudnason V, Gyllensten U, Hamsten A, Hattersley AT, Hofman A, Hu FB, Illig T, Iribarren C, Jarvelin MR, Kao WH, Kaprio J, Launer LJ, Munroe PB, Oostra B, Penninx BW, Pramstaller PP, Psaty BM, Quertermous T, Rissanen A, Rudan I, Shuldiner AR, Soranzo N, Spector TD, Syvanen AC, Uda M, Uitterlinden A, Völzke H, Vollenweider P, Wilson JF, Witteman JC, Wright AF, Abecasis GR, Boehnke M, Borecki IB, Deloukas P, Frayling TM, Groop LC, Haritunians T, Hunter DJ, Kaplan RC, North KE, O'Connell JR, Peltonen L, Schlessinger D, Strachan DP, Hirschhorn JN, Assimes TL, Wichmann HE, Thorsteinsdottir U, van Duijn CM, Stefansson K, Cupples LA, Loos RJ, Barroso I, McCarthy MI, Fox CS, Mohlke KL, Lindgren CM; MAGIC. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet*. 2010;42:949–960. doi: 10.1038/ng.685
20. Neale Lab. Rapid GWAS of thousands of phenotypes for 337,000 samples in the UK Biobank. 2017. http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank. Accessed December 17, 2017.
21. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013;45:580–585.
22. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun*. 2017;8:1826. doi: 10.1038/s41467-017-01261-5
23. Pers TH, Karjalainen JM, Chan Y, Westra HJ, Wood AR, Yang J, Lui JC, Vedantam S, Gustafsson S, Esko T, Frayling T, Speliotes EK, Boehnke M, Raychaudhuri S, Fehrmann RS, Hirschhorn JN, Franke L; Genetic Investigation of ANthropometric Traits (GIANT) Consortium. Biological interpretation of genome-wide association studies using predicted gene functions. *Nat Commun*. 2015;6:5890. doi: 10.1038/ncomms6890
24. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet*. 2009;41:677–687. doi: 10.1038/ng.384
25. Graef IA, Chen F, Chen L, Kuo A, Crabtree GR. Signals transduced by Ca(2+)/calcineurin and NFATc3/c4 pattern the developing vasculature. *Cell*. 2001;105:863–875.
26. Fukuda S, Tomatsu S, Masue M, Sukegawa K, Iwata H, Ogawa T, Nakashima Y, Hori T, Yamagishi A, Hanyu Y. Mucopolysaccharidosis type IVA: N-acetylgalactosamine-6-sulfate sulfatase exonic point mutations in classical Morquio and mild cases. *J Clin Invest*. 1992;90:1049–1053. doi: 10.1172/JCI115919
27. Putnam EA, Zhang H, Ramirez F, Milewicz DM. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. *Nat Genet*. 1995;11:456–458. doi: 10.1038/ng1295-456
28. Lee AJ, Evans CJ, Allan PL, Ruckley CV, Fowkes FG. Lifestyle factors and the risk of varicose veins: Edinburgh Vein Study. *J Clin Epidemiol*. 2003;56:171–179.
29. Ciardullo AV, Panico S, Bellati C, Rubba P, Rinaldi S, Iannuzzi A, Cioffi V, Iannuzzo G, Berrino F. High endogenous estradiol is associated with increased venous distensibility and clinical evidence of varicose veins in menopausal women. *J Vasc Surg*. 2000;32:544–549. doi: 10.1067/mva.2000.107768
30. Scott TE, Mendez MV, LaMorte WW, Cupples LA, Vokonas PS, Garcia RI, Menzoian JO. Are varicose veins a marker for susceptibility to coronary heart disease in men? Results from the Normative Aging Study. *Ann Vasc Surg*. 2004;18:459–464. doi: 10.1007/s10016-004-0056-z
31. Abramson JH, Hopp C, Epstein LM. The epidemiology of varicose veins: a survey in western Jerusalem. *J Epidemiol Community Health*. 1981;35:213–217.
32. Komsuoğlu B, Göldeli O, Kulan K, Cetinarslan B, Komsuoğlu SS. Prevalence and risk factors of varicose veins in an elderly population. *Gerontology*. 1994;40:25–31. doi: 10.1159/000213571

33. Malhotra SL. An epidemiological study of varicose veins in Indian railroad workers from the South and North of India, with special reference to the causation and prevention of varicose veins. *Int J Epidemiol*. 1972;1:177–183.
34. Hirai M, Naiki K, Nakayama R. Prevalence and risk factors of varicose veins in Japanese women. *Angiology*. 1990;41:228–232. doi: 10.1177/000331979004100308
35. Carpentier PH, Maricq HR, Biro C, Ponçot-Makinen CO, Franco A. Prevalence, risk factors, and clinical patterns of chronic venous disorders of lower limbs: a population-based study in France. *J Vasc Surg*. 2004;40:650–659. doi: 10.1016/j.jvs.2004.07.025
36. Khalil SF, Mohktar MS, Ibrahim F. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. *Sensors (Basel)*. 2014;14:10895–10928. doi: 10.3390/s140610895
37. Lindholm D, Fukaya E, Leeper NJ, Ingelsson E. Bioimpedance and new-onset heart failure: a longitudinal study of >500 000 individuals from the general population. *J Am Heart Assoc*. 2018;7:e008970.
38. Anwar MA, Georgiadis KA, Shalhoub J, Lim CS, Gohel MS, Davies AH. A review of familial, genetic, and congenital aspects of primary varicose vein disease. *Circ Cardiovasc Genet*. 2012;5:460–466. doi: 10.1161/CIRCGENETICS.112.963439
39. Li J, Hou B, Tumova S, Muraki K, Bruns A, Ludlow MJ, Sedo A, Hyman AJ, McKeown L, Young RS, Yuldasheva NY, Majeed Y, Wilson LA, Rode B, Bailey MA, Kim HR, Fu Z, Carter DA, Bilton J, Imrie H, Ajuh P, Dear TN, Cubbon RM, Kearney MT, Prasad RK, Evans PC, Ainscough JF, Beech DJ. Piezo1 integration of vascular architecture with physiological force. *Nature*. 2014;515:279–282. doi: 10.1038/nature13701
40. Zamboni P, Izzo M, Tognazzo S, Carandina S, De Palma M, Catozzi L, Caggiati A, Scapoli G, Gemmati D. The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. *Free Radic Biol Med*. 2006;40:1869–1873. doi: 10.1016/j.freeradbiomed.2006.01.026
41. Brown K, Luddington R, Taylor SA, Lillicrap DP, Baglin TP. Risk of venous thromboembolism associated with the common hereditary haemochromatosis Hfe gene (C282Y) mutation. *Br J Haematol*. 1999;105:95–97.
42. Le Flem L, Mennen L, Aubry ML, Aiach M, Scarabin PY, Emmerich J, Alhenc-Gelas M. Thrombomodulin promoter mutations, venous thrombosis, and varicose veins. *Arterioscler Thromb Vasc Biol*. 2001;21:445–451.
43. Sverdlova AM, Bubnova NA, Baranovskaya SS, Vasina VI, Avitisjan AO, Schwartz EI. Prevalence of the methylenetetrahydrofolate reductase (MTHFR) C677T mutation in patients with varicose veins of lower limbs. *Mol Genet Metab*. 1998;63:35–36. doi: 10.1006/mgme.1997.2638
44. Poredos P, Spirkoska A, Rucigaj T, Fareed J, Jezovnik MK. Do blood constituents in varicose veins differ from the systemic blood constituents? *Eur J Vasc Endovasc Surg*. 2015;50:250–256. doi: 10.1016/j.ejvs.2015.04.031
45. Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. *N Engl J Med*. 2006;355:488–498. doi: 10.1056/NEJMra055289
46. Darvall KA, Sam RC, Adam DJ, Silverman SH, Fegan CD, Bradbury AW. Higher prevalence of thrombophilia in patients with varicose veins and venous ulcers than controls. *J Vasc Surg*. 2009;49:1235–1241. doi: 10.1016/j.jvs.2008.12.017
47. Zoller B, Ji J, Sundquist J, Sundquist K. Venous thromboembolism and varicose veins share familial susceptibility: a nationwide family study in Sweden. *J Am Heart Assoc*. 2014;3:e000850.
48. Pennisi D, Gardner J, Chambers D, Hosking B, Peters J, Muscat G, Abbott C, Koopman P. Mutations in So×18 underlie cardiovascular and hair follicle defects in ragged mice. *Nat Genet*. 2000;24:434–437. doi: 10.1038/74301
49. Briegel KJ, Joyner AL. Identification and characterization of Lbh, a novel conserved nuclear protein expressed during early limb and heart development. *Dev Biol*. 2001;233:291–304. doi: 10.1006/dbio.2001.0225
50. Winslow MM, Pan M, Starbuck M, Gallo EM, Deng L, Karsenty G, Crabtree GR. Calcineurin/NFAT signaling in osteoblasts regulates bone mass. *Dev Cell*. 2006;10:771–782. doi: 10.1016/j.devcel.2006.04.006
51. Zoller B, Ji J, Sundquist J, Sundquist K. Body height and incident risk of venous thromboembolism: a cosibling design. *Circ Cardiovasc Genet*. 2017;10:e001651.