Clinical and Genetic Determinants of Varicose Veins

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Varicose veins

- 30 million affected adults in the US
- >$1 billion annual healthcare costs
- Ulceration in up to 20% of affected individuals
- May be associated with DVT or even PAD

Risk factors for varicose veins

The epidemiology of varicose veins: the Framingham Study.

- Age, Female sex, Obesity, Pregnancy, History of DVT

subjects who had ever smoked for over a year, and of these heavier smokers (≥15 cigarettes a day), had higher risk of varicose veins, IORs 1.3 (95% CI: 0.9–1.8) and 1.8 (95% CI: 1.1–2.8) respectively. Daily use of meat seemed to decrease the risk of

patients with VV (49.4%). Other factors, such as congestive heart failure, angina pectoris, hypertension, cigarette smoking, diabetes mellitus, height, weight, obesity, or hyperlipidemia, were not found to be associated with the prevalence of VV.

Risk factors for varicose veins

- Age, Female sex, Obesity, Pregnancy, History of DVT.

Family history
Strong genetic component

Varicose veins

- A common condition with an unclear pathogenesis
- Increasingly been associated with serious health risks, including DVT
- Lack of insight into pathogenesis may help explain why no approved therapies exist

Define the clinical and genetic determinants of varicose vein disease
Methods

- Study population: UK Biobank, a longitudinal cohort study of over 500,000 individuals from the United Kingdom

- Observational analyses
  - Machine learning
  - Traditional epidemiological analyses

- Genetic studies
  - Genome-wide association study (GWAS)
  - Pathway analysis
  - Mendelian randomization (MR) analysis

Epidemiology and environmental risk factors

Causal genetic and biological associations
Study cohort

- 502,619 participants

- 11,541 cases (2,441 incident)
- Median follow-up: 6.2 y

Methods – observation analyses

- Traditional epidemiological approaches
- Machine learning

*Hypothesis free search for risk factors*

**GRADIENT BOOSTING MACHINE MODEL**

Validated with traditional Cox regression

2715 predictor variables
Methods – genetic studies

- First genome-wide association study (GWAS) for varicose veins
- Pathway and functional analysis
OBSERVATIONAL STUDIES
Study flowchart

502,619 participants
22 centers across the United Kingdom
2006-2010

Observational analyses
Exclusion of individuals with prior diagnosis of varicose veins (by ICD code; n = 9,100)

493,519 individuals
2,441 incident events
Median follow-up: 6.2 years

Genome-wide association study
Available phenotype data
Exclusion of related individuals or those of non-European ancestry (n = 1,964)

337,536 individuals
9,577 varicose vein cases
Established risk factors
Debated risk factors

Variable | Age- and sex-adjusted HR (95%CI) | Fully adjusted** HR (95%CI)
---|---|---
Smoking | 1.04 (0.92-1.19) | 1.04 (0.91-1.19)
Oral contraceptive use | 0.67 (0.44-1.02) | 0.70 (0.45-1.07)

Low importance in machine learning

No significant associations in multivariable analysis

Fully adjusted model includes age, gender, history of DVT, BMI, waist-hip ratio, and pregnancy.
Machine learning

- Traditional risk factors again observed (DVT, pregnancy-related)
- Several novel predictors of varicose veins identified

- Age deep-vein thrombosis diagnosed
- Bioimpedance of leg (left)
- Had other major operations
- Surgery on leg arteries
- Standing height
- Ulcer of lower limb (ICD-10: L97)
- Age at first live birth
- Bioimpedance of leg (right)
- Other soft tissue disorders (ICD-10: M79)
- Birth weight of first child

Variable importance
Novel link to height

Probability of varicose veins

Height (cm)

p < 0.0001
Validated findings from machine learning

Most predictive variables:
- Leg bioimpedance
- Height

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age- and sex-adjusted HR (95%CI)</th>
<th>Fully adjusted HR (95%CI)</th>
<th>C-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novel</td>
<td></td>
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</tr>
<tr>
<td>Leg bioimpedance</td>
<td>0.56 (0.51-0.63)</td>
<td>0.43 (0.39-0.50)</td>
<td>0.645</td>
</tr>
<tr>
<td>Height</td>
<td>1.70 (1.48-1.96)</td>
<td>1.74 (1.51-2.01)</td>
<td>0.610</td>
</tr>
<tr>
<td>Major operations</td>
<td>0.75 (0.69-0.82)</td>
<td>0.77 (0.70-0.84)</td>
<td>0.584</td>
</tr>
<tr>
<td>Surgery on leg arteries</td>
<td>3.71 (2.46-5.62)</td>
<td>3.50 (2.30-5.33)</td>
<td>0.583</td>
</tr>
<tr>
<td>Other soft tissue disorders (ICD-10: M79)</td>
<td>2.01 (1.58-2.55)</td>
<td>1.66 (1.30-2.12)</td>
<td>0.578</td>
</tr>
<tr>
<td>Cellulitis (ICD-10: L03)</td>
<td>2.09 (1.52-2.87)</td>
<td>1.79 (1.29-2.48)</td>
<td>0.577</td>
</tr>
<tr>
<td>Ulceration of lower limb (ICD-10: L97)</td>
<td>4.65 (2.36-9.16)</td>
<td>3.00 (1.36-6.61)</td>
<td>0.575</td>
</tr>
</tbody>
</table>

Fully adjusted model includes age, gender, history of DVT, BMI, waist-hip ratio, and pregnancy.
GENETIC STUDIES
GWAS of 330,000 individuals

30 novel genome-wide significant loci
eQTL, pathway, and functional analyses

**VV SNPs**
- **rs2861819**
  - **PNO1, WDR92, PLEK, PPP3RI**
  - Signaling during angiogenesis (calcineurin)
- **rs20911463**
  - **PIEZ01**
  - Vascular mechanosensory channel
- **rs3101725**
  - **GALNS**
  - Linked to:
    - Hereditary skeletal dysplasia
    - Marfan-like syndrome
    - Congenital contractural arachnodactyly
  - Enrichment for pathways in angiogenesis and limb biology/height

**Pathways**
- **vascular biology**
- **skeletal/limb biology**
IS THERE A CAUSAL RELATIONSHIP?
Mendelian Randomization (MR)

SNPs → Reliable association → Exposure → Disease

No association

No independent association

Mendelian Randomization (MR)

Genetic variants associated with modifiable exposure

SNP\(_1\)  SNP\(_2\)  SNP\(_x\)

\[\text{Modifiable exposure}\]

\[\text{Disease}\]
Mendelian Randomization (MR)

Genetic variants associated with height

SNP_1  SNP_2  SNP_x

Height

Varicose veins

Genetic correlation and Mendelian Randomization (MR)

- Integrate data from our GWAS and GWAS for height
  - GIANT study: **Genetic Investigation of ANthropometric Traits**

SNPs 512 Height SNPs
Genetic correlation and Mendelian Randomization (MR)

- Integrate data from our GWAS and GWAS for height
  - GIANT study: Genetic Investigation of ANthropometric Traits

Strong genetic correlation between height and varicose veins

### MR analysis

<table>
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<tr>
<th>Method</th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Conventional MR (inverse-variance weighted)</td>
<td>9,577</td>
<td>327,959</td>
<td>1.26 (1.24-1.28)</td>
</tr>
<tr>
<td>Maximum likelihood</td>
<td>9,577</td>
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### MR excluding heterogenous variants

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<th>P-value</th>
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<td>Maximum likelihood</td>
<td>512</td>
<td>1.26</td>
<td>2.07E-16</td>
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<td>Inverse-variance weighted</td>
<td>512</td>
<td>1.28</td>
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<tr>
<td>Weighted median</td>
<td>512</td>
<td>1.26</td>
<td>9.12E-18</td>
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Conclusions

- Largest and most comprehensive study of varicose veins to date
- Defined clinical determinants of disease
- Established height as novel risk factor
- Identified genetic risk loci in the first GWAS of varicose veins
  - May represent new translational targets

Acknowledgements

Circulation

EDITORIAL

Varicose Veins Reach New Heights

- Stanford
  - Eri Fukaya, MD, PhD
  - Alyssa Flores, BS
  - Daniela Zanetti, PhD
  - Erik Ingelsson, MD, PhD
  - Nicholas Leeper, MD

- Uppsala University, Sweden
  - Daniel Lindholm, MD, PhD
  - Stefan Gustafsson, PhD
Limitations

- Incidence of varicose veins defined by ICD code
- Generalizability
  - UK Biobank - mostly white, middle-aged adults
- Difficulty in capturing candidate predictor variables
  - Physical activity
  - Diet
Height and varicose veins

- Novel predictor in machine learning
- Independently associated in multivariable-adjusted Cox regression
- Varicose vein loci enriched for height-associated pathways

Next sought to determine whether height is a causal risk factor for varicose veins
Mendelian randomization (MR)

- Power for MR: 100%
  - 13.0% variance explained
  - Effect size 1.51 per SD increase in height

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