A Large Scale Genome Wide Association Study of Varicose Veins in the 23andMe Cohort

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Introduction

- Varicose veins are a common chronic condition in which veins, usually in the legs, become enlarged and twisted due to deficient functioning of one-way valves that normally return blood to the heart.
- While varicose veins are most commonly considered to be a cosmetic problem, in some cases they can lead to, or signal, more serious circulatory problems under the general classification of chronic venous incompetence (CVI). Other symptoms of CVI include pain, edema, swelling, hyperpigmentation and ulceration.
- Estimates of prevalence of varicose veins range from approximately 5% to 30% in adults with prevalence in females about three times as high as males, although at least one study reports slightly elevated risk in males rather than females[1,2].

Methods

Phenotype Data Collection

- Participants were drawn from the customer base of 23andMe, Inc., a consumer genetics company. Participants provided informed consent and participated in the research online. Participants answered the question : “Do you have varicose veins on your legs? Yes/No/I’m not sure”.
- The sample consisted of 92,666 23andMe customers of European descent, filtered to remove close relatives. A total of 23,565 (25.4%) responded “yes” and 69,101 (74.6%) responded “no”. The 47,340 women were roughly three times more likely to report varicose veins as the 45,326 men (36.8% versus 13.5%). Prevalence varied by age: respondents under 30 reported “yes” just 11.8% of the time, compared to 26.9% for age 30-45 and increasing to 33.2% for those over 60.

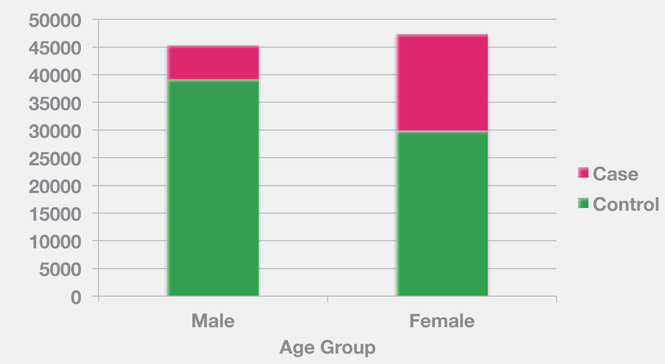

Figure 1. Distribution of survey responses by sex.

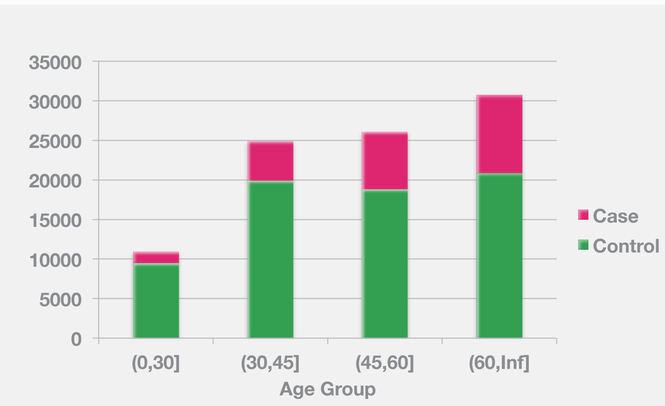

Figure 2. Distribution of survey responses by age.

Statistical Analysis

- We conducted a genome wide association test using approximately 950K genotyped SNPs and 13.7M imputed SNPs.
- We performed logistic regression assuming an additive model for allelic effects, and controlling for age, sex, and population structure (first five principal components) using the model:

varicose_veins_legs ~ age + sex + pc.0 + pc.1 + pc.2 + pc.3 + pc.4 + genotype

- p-values for SNPs were calculated using likelihood ratio tests for logistic regressions. The results in this report have been adjusted for a genomic control inflation factor λ=1.074

Acknowledgments

- We thank 23andMe customers who consented to participate in research for enabling this study. We also thank employees of 23andMe who contributed to the development of the infrastructure that made this research possible.
- This work was supported in part by the National Human Genome Research Institute of the National Institutes of Health under grant number 2R44HG006981-02..

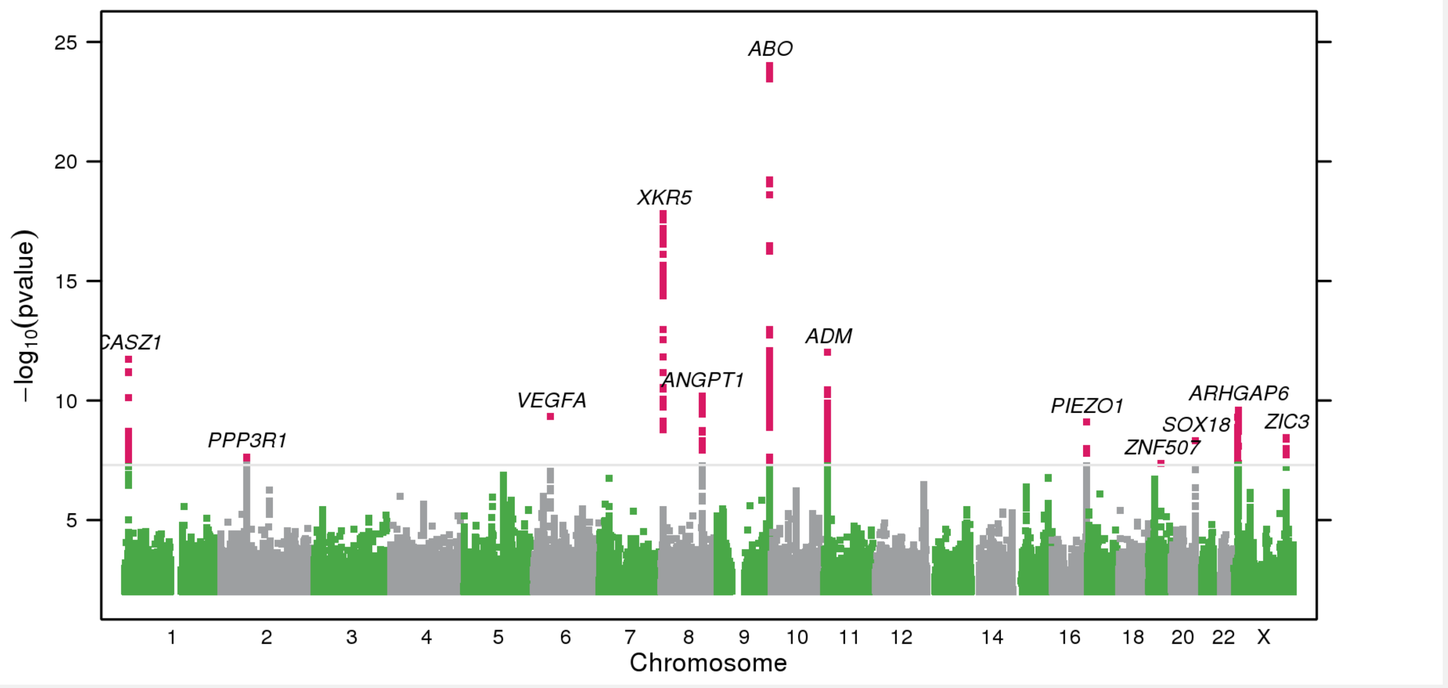

Figure 3. Manhattan plot depicting genome wide view of SNP associations with varicose veins in the leg. SNPs shown in red are genome-wide significant (p < 5e-8). Regions are named with the postulated candidate gene.

Region	SNP	Position	Alleles	p value	OR	95% CI	Gene Context
9q34.2	rs507666	136149399	A/G	9.3×10^{-20}	0.862	[0.838,0.887]	[ABO]
8p23.1	rs966562	6655358	A/T	1.5×10^{-16}	0.900	[0.879,0.921]	AGPAT5--[]--XKR5
11p15.4	rs7111987	10353717	A/G	9.5×10^{-13}	0.917	[0.895,0.939]	ADM--[]---AMPD3
1p36.22	rs11121615	10825577	C/T	1.8×10^{-12}	0.910	[0.887,0.934]	[CASZ1]
8q23.1	rs111434909	108387224	C/T	6.5×10^{-11}	0.907	[0.880,0.934]	[ANGPT1]
Xp22.2	rs145218303	11591173	G/T	2.4×10^{-10}	1.086	[1.059,1.114]	[ARHGAP6]
6p21.1	rs6905288	43758873	A/G	4.7×10^{-10}	1.076	[1.051,1.101]	VEGFA-[]---C6orf223
16q24.3	rs4516218	88793090	A/G	8.0×10^{-10}	0.898	[0.868,0.930]	[PIEZO1]
Xq26.3	rs4463578	136767331	C/T	3.6×10^{-9}	1.069	[1.046,1.093]	ZIC3---[]---FGF13
20q13.33	rs6062618	62682529	G/T	4.9×10^{-9}	1.081	[1.053,1.110]	SOX18-[]--TCEA2
2p14	rs6712038	68492887	A/G	2.3×10^{-8}	0.930	[0.906,0.954]	PPP3R1--[]--CNRIP1
19q13.11	rs79607156	32703618	A/T	4.3×10^{-8}	0.782	[0.715,0.855]	THEG5---[]---ZNF507

Table 4. Index SNPs for regions under p = 5 x 10^{-8} . The index SNP is defined as the SNP with the smallest p-value within a region; or the SNP with the smallest p-value in the conditional analysis.

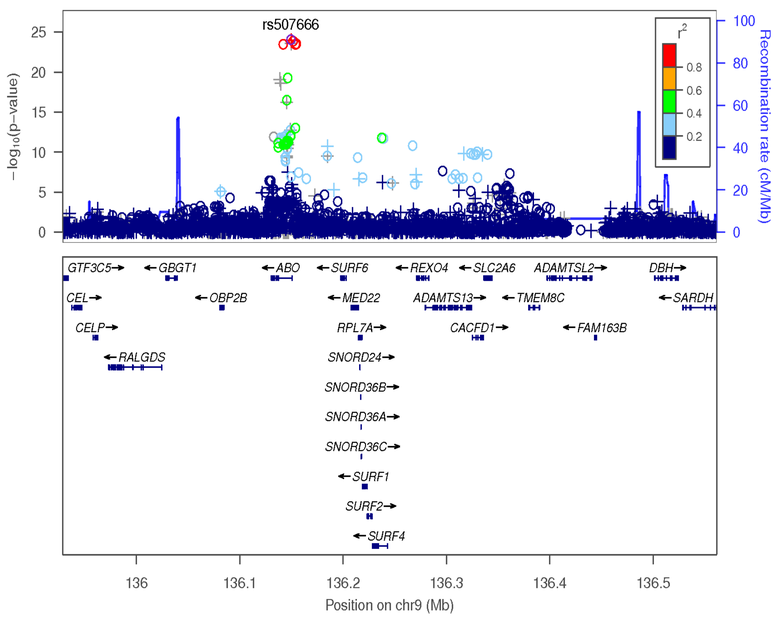

Figure 5. Details of the region around rs507666. Colors depict the squared correlation (r2) of each SNP with the most associated SNP (which is shown in purple). Gray indicates SNPs for which r2 information was missing.

References

1. Eberhardt, R. T. and Raffeto, J. D. Chronic Venous Insufficiency. *Circulation*. 2005; 111; 2398-2409
2. Evans CJ et al. Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. *J Epidemiol Community Health*. 1999; 53: 149–153.
3. Cornu-Thenard et al. Relationship between blood groups (ABO) and varicose veins of the lower limbs. A case-control study. *Phlebology* 1989 4, 37-40

Results

We found twelve significant novel associations of SNPs with varicose veins (p < 5 x 10^{-8}). Figure 3 and Table 4 summarize our findings.

- Our strongest association was with SNP rs507666 (p=5.2e-20), found in ABO on chromosome, which encodes a protein defining the ABO blood group. Figure 5 shows details of the region surrounding rs507666. Variation in SNP rs507666 determines A1 blood subgroup status. This finding is consistent with a previous study[3] that reported the association between the A blood group and varicose veins. It also identified an additional SNP, rs966562 (p=1.8e-16), near XKR5 involved in Kell blood group determination.
- Our study also identified SNPs in genes which have previously been characterized as players in the regulation of blood pressure or circulation, including SNP rs11121615 (p=8.1e-12) in CASZ1, which promotes vascular development and is associated with blood pressure variation, and SNP rs7111987 (p=2.2e-10) near ADM which is a potent, long-lasting vasodilator peptide, and also inhibits apoptosis and promotes angiogenesis. SNP rs145218303 is in ARGHAP6 which encodes protein that up regulates PLC-delta1, which shows increased activity in hypertension
- The GWAS of varicose veins also points to a role of genes involved in circulatory development. In addition CASZ1 to ADM mentioned above: SNP rs1433196 (p=5.2e-11) is found in ANGPT1 that encodes a type of angiopoietin, a group of proteins with important roles in vascular development; SNP rs966562 (mentioned above) near ANGPT2, that encodes an antagonist for ANGPT1; and SNP rs6905288 (p=1.5e-8) near VEGFA which encodes a protein whose effects include angiogenesis, vasculogenesis and endothelial cell growth. Finally, rs4516218 is in PIEZO1, which encodes an ion channel thought to play a role in adult cardiovascular function and disease.

Discussion

- This is the first GWAS examining varicose veins. The results of this study provide some interesting insights into the nature and causes of varicose veins. The association with the ABO gene and Kell blood group suggest an inherited susceptibility.
- Associations with the function of the circulatory system may indicate either a role in causing symptoms, or in the ability of the body to compensate for, or repair vascular incompetence.
- Finally associations with genes involved in angiogenesis may provide some indications of the mechanisms underlying the defective valves that cause varicose veins, either through the inability to produce properly formed valves or to repair defects during construction.