Evidence for a genetic role in varicose veins and chronic venous insufficiency

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Abstract
There is a strong body of circumstantial evidence which implicates genetics in the aetiology and pathology of varicose veins and venous ulcer disease. The aim of this review is to consider the current knowledge of the genetic associations and the ways in which new genetic technologies may be applied to advancing our understanding of the cause and progression of these venous diseases. A number of publications have used a candidate gene approach to identify genes implicated in venous disease. Although these studies have opened up important new insights, there has been a general failure to replicate results in an independent cohort of patients. With our limited knowledge of the biological pathways involved in the pathogenesis of venous disease we are not in a strong position to formulate truly erudite a priori candidate gene hypothesis-directed studies. A genome-wide association study should therefore be considered to help further our understanding of the genetic basis of venous disease. Due to the large sample sizes required for discovery and validation, using the new generations of molecular technologies, it will be necessary to form collaborating groups in order to successfully advance the field of venous disease genetics.

Keywords: varicose veins; venous; genome-wide scan; genetics

Introduction
Chronic venous disease (CVD) is common and it is well recognized that it runs in families. The exact nature of the genetic basis for this association is, however, far from clear. There have been a number of different approaches aimed at unraveling this question. Despite a strong body of circumstantial evidence which implicates genetic influences in the aetiology and pathology of these venous diseases, there remains a significant amount of confusion. The recent advancements in the knowledge of gene structure and function have been striking; however, their application to venous disease is very limited. Genetic technologies and their use in clinical studies are multiplying at a great rate for many vascular diseases, but this is not the case for venous disease.

The aim of this review is to consider the current knowledge of the genetic associations with venous disease and the ways in which new genetic technologies may be applied to advancing our understanding of the cause and progression of varicose vein disease. These technologies may also lead to the recognition of new critical metabolic pathways and approaches to inhibit the development and treatment of varicose veins. For example, they may not only further our understanding of the effects of phlebotonic drugs, such as flavonoids and horse-chestnut extracts, but may also suggest other pharmacological strategies.

There are a number of approaches that have been taken to evaluate the genetic contribution to varicose vein disease. These include heritability analysis through family studies, differential gene expression studies and studies of genomic variation, either by comparing individual candidate

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Family studies

Family-based studies have been the cornerstone of establishing the familial risk and heritability of human disease. Positive family history in patients with venous disease has been reported as early as 1855. Brinsuk et al. compared venous function between 24 monozygotic and 22 dizygotic twins and concluded that venous function is strongly influenced by genetic factors.

There is a wide discrepancy in the quoted prevalence of varicosities in patients with positive family history, ranging between 6% and 80%. In 1994, Cornu-Thenard et al. re-examined the role of the familial factor in varicose disease in a prospective case-control study. They showed that the risk of developing varicose veins was 90% for the children when both parents had varicose veins, 25% for men and 62% for women when one parent was affected, and 20% when neither parent was affected. This suggested an autosomal-dominant inheritance with variable penetrance. A Chinese analysis of nuclear families reported penetrance of between 70% and 92%, while 37% of their cases were sporadic. A Finnish longitudinal study showed a 1.6-fold increased risk of developing varicose veins in those with a family history of varicosities. Fiebig et al. examined heritability of CVD and concluded that the additive genetic component was approximately 17%.

These studies suggest a strong genetic component in primary venous failure but the genes which are involved are yet to be identified.

Gene expression studies

One approach to the identification of genes that might be implicated in the development of varicose veins is to examine the expression of specific genes in the diseased tissue. This is done by comparing RNA from varicose and normal veins. A number of studies have been published which demonstrate a diversity of genes which are differentially regulated in varicose vein tissues. Major functional classes of genes expressed differentially in varicose veins and ulcers have involved metabolic pathways, extracellular matrix organization and regulation, response to external stimuli and cell organization. Saphenofemoral junction incompetence has also been shown to be associated with altered transcription of vascular endothelial growth factor and its receptors.

While this appears to be a logical approach, interpretation of such gene expression studies can be difficult. Tissue RNA levels provide a temporal snapshot of expression patterns and it can be difficult to determine whether differentially expressed transcripts are a cause or consequence of the disease phenotype.

Changes in local RNA expression of a gene may be driven by both environmental and genetic mechanisms. The expression of the promoter region of a gene can be modified by regulatory elements elsewhere in the same gene, as well as by signalling from other genes. This is complicated by the gene-modifying effects of microRNAs and the phenomena of epigenetics. Gene expression information, in itself, is therefore not sufficient to fully elucidate the relationship between genome sequence, gene expression and cellular dynamics. Furthermore, the common approach of many studies of looking for genetic polymorphic variation primarily within the promoter regions of genes showing differential RNA expression is a limited strategy.

In this review we have therefore focused on publications which examined genome sequence rather than gene expression.

An electronic search was conducted to identify reports of published studies relating to the genetics of venous disease. Databases searched included Medline (1 January 1966 to 1 November 2009) and Embase (1 January 1980 to 1 November 2009). There were no restrictions in terms of language or publication year. The following search terms were used: ‘genetics’, ‘genome’, ‘gene’ and ‘varicose veins’. This identified 467 articles, 94 of which examined a genetic role in varicose veins and CVD. Only 15 of those examined candidate genes in venous disease. The remainder included gene expression and family studies. The number of publications was insufficient to evaluate the different types of venous disease as separate entities. We therefore looked at a spectrum of disease which included primary varicose veins and their sequelae, including venous ulcer disease. Studies which specifically looked at thromboembolic venous disease were excluded.
Candidate gene approach

A candidate gene approach to identifying the genes associated with venous disease is to examine those genes for which there is a reasonable likelihood of involvement. This would be based on prior assumptions, for example, looking for variation in a gene known to influence a key relevant enzyme or protein structure or critical pathway. This strategy is potentially useful in conditions in which underlying pathological pathways are well understood. For example, this approach was successfully used in studies which looked at the role of the insulin gene in type 1 diabetes mellitus. In complex diseases, where the underlying pathways are usually less clear, the candidate gene approach has met with mixed success as there are so many possible candidates that it becomes a very inefficient process, much like looking for a needle in a haystack.

Some studies using a candidate gene approach to investigate venous disease genetics have been carried out. These studies have opened up important new insights but they also illustrate the limitations of the candidate approach.

Varicose veins

FOXC2 was the first gene described in which mutations were strongly associated with primary venous valve failure in both the superficial and deep veins of the lower limb. Brice et al. showed that the single FOXC2 gene mutation was the cause of the rare inherited condition of lymphoedema distichiasis. This is a condition in which varicose veins are a common feature. They hypothesized that this gene might also be implicated in the development of varicose veins in the normal population. By studying 2060 female twin pairs they concluded that there was a functional variant within the vicinity of the FOXC2 gene which predisposed individuals to varicose veins. A further study by these workers showed that every participant (n = 18) with the FOXC2 mutation had evidence of venous reflux on duplex ultrasound scanning, compared with one in 12 controls (P < 0.0001). The valves in the superficial veins were always affected by the FOXC2 mutation and in 78% of the deep system. This is an example in which a rare monogenic mutation has provided a candidate gene for further study in the more general polygenic condition of varicose veins. Unfortunately, there have been no further studies confirming an association of FOXC2 with varicose veins.

Chronic venous insufficiency

The ‘hemochromatosis gene’ mutation has become to be associated with venous ulcers through the insightful work of Zamboni et al. They examined the hemochromatosis gene (HFE) C282Y mutation and its association with venous leg ulceration based on the knowledge that CVD leads to local iron overload in the area of skin changes in the affected leg. A case-control study design was used to investigate whether HFE mutations would increase the risk of chronic venous leg ulceration. The study cohort consisted of 238 patients with CVD, based on clinical and duplex examinations, and 280 controls taken from among blood donors. CVD patients were subdivided into those with and without skin lesions. Within the CVD
population the C282Y mutation was significantly associated with the ulcer subgroup. The authors concluded that the presence of the C282Y mutation would strengthen the indications for surgical correction of superficial venous insufficiency.26 It is important to note that the C282Y allele frequency was not altered in patients with varicose veins without ulcer disease. In fact, the prevalence of the allele in these patients was less than in the controls but this did not reach statistical significance. This suggests that the C282Y variant has more relevance to ulcer biology than to the development of varicose veins.

The same group also investigated the factor XIII V34L gene polymorphism. Low-factor XIII activity has been reported in the blood of patients with venous ulceration, while topical factor XIII treatment appears to improve venous ulcer healing.29,30 They studied a group of 91 patients with venous ulceration (61 primary, 24 post-thrombotic and 6 mixed aetiology) and compared them with 195 blood donor controls. Although no significant difference was found in the factor XIII genotype frequencies in cases and controls, they did find a relationship with ulcer size.31 A further study of 91 patients (with the exclusion of those with post-thrombotic syndrome) showed that the factor XIII-34L variant was significantly associated with shorter healing time after superficial venous surgery, suggesting a role in the healing and tissue regeneration phases.32

Polymorphic variants of the oestrogen and tumour necrosis factor (TNF) genes have also been implicated in venous ulceration. Oestrogen has been shown to improve the rate of wound healing.33,34 Ashworth et al. examined genetic polymorphic variation in the promoter region of oestrogen receptor-B (ERB) in 125 venous ulcer cases, with venous insufficiency confirmed by Doppler ultrasound assessment, compared with 380 venous ulcer-free controls. They observed altered polymorphism frequencies flanking 5' regulatory elements and concluded that ERB played a regulatory role in both inflammatory and repair processes.35

TNF-A polymorphisms have been associated with inflammatory conditions.36 Leg ulcer wound fluid levels of TNF-A decrease as ulcers start to heal.37 Wallace et al. conducted a case-control study comparing the frequency of gene polymorphisms in the promoter of the TNF-A gene in 181 patients with venous ulcers and 181 controls. Controls were excluded if they had abnormal venous function on photoplethysmography. They found two-fold increase in the risk of ulceration seen in carriers of the TNFA-308A allele.38

All these studies, whether for the HFE C282Y, factor XIII V34L, ERB or TNFA-308A, flag the possibility that genetic markers may be associated with CVD clinical severity progression but not necessarily with primary varicose vein susceptibility. These observations also demonstrate the importance of the clarity in disease phenotype selection and definition in the design of association studies, and their importance for subsequent study comparisons and meta-analysis.

It has also been suggested that patients with venous ulceration have an increased prevalence of genetic thrombophilia, similar to that observed in patients with deep vein thrombosis.39–41 Darvall et al.42 compared the prevalence of thrombophilia in 27 patients with varicose veins and 27 patients with chronic venous ulceration (those with a history of or risk factor for deep vein thrombosis were excluded) with a control group (n = 54) without clinical and duplex evidence of venous disease. The principle finding was that patients with varicose veins had a much higher prevalence of thrombophilia than the controls (67% vs. 37%), as did the chronic venous ulcer group (74% vs. 33%). Candidate genes for chronic venous insufficiency are listed in Table 2.

Limitations of candidate gene approach

While the candidate approach is intuitively sensible and seemingly simple, the successful identification and reliable validation of a genetic variation associated with venous disease has not yet been achieved. In 2003, Pistorius looked at epidemiological and genetic aspects of the heredity of varicose veins. At that time, he stated that ‘the majority of studies identified in the literature suffer from huge methodological biases linked to the method of data collection and to nosological inaccuracies’.43 Unfortunately, very little has changed in the intervening years.

In the field of genetic association for complex biological traits, these study limitations have been known for well over a decade and have led

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<th>Table 2 Candidate genes for chronic venous insufficiency</th>
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<td>Gene</td>
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<tr>
<td>Haemochromatosis26–28</td>
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<tr>
<td>Factor XIII V34L29,30</td>
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<td>Oestrogen33,34</td>
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<td>Tumour necrosis factor36–38</td>
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to best-practice guidelines emphasizing the features of: ‘large sample sizes, small \( P \) values which include correction for multiple testing and biological (mechanistic pathway or functional) plausibility’. There should be an initial study, followed by independent replication, preferably within multiple cohorts.\(^{44}\) In addition, there should be ‘meticulous characterization of the study groups’\(^{45}\) including the controls who should be shown to be free of the disease phenotype.

When comparing venous genetic association studies that have been conducted to date, and as described above, none have fulfilled these best-practice standards particularly well. While their \textit{a priori} hypotheses appear reasonable there is a general failure of producing replicate results in independent cohorts. In addition, they examine groups of patients, each defined differently, some based on clinical examination while others had a duplex scan. The quality of controls is also an issue, most being unscreened for the phenotype, introducing a strong false-negative bias as a significant proportion of these controls may have had superficial venous disease. Finally, the vast majority of the reported studies have been statistically underpowered. All of this makes it difficult to draw any strong conclusions from the body of genetic association studies conducted on varicose veins and its sequelae to date.

Clearly, better candidate studies are required. However, with our limited knowledge of the biological pathways involved in the pathogenesis of venous disease, we are not in a strong position to formulate truly erudite \textit{a priori} candidate gene hypothesis-directed studies. There have been other advances in genetic association studies beyond the use of a hypothesis-driven candidate approach. It is now possible to examine genomic variation without having to focus on individual genes, sometimes called a hypothesis-free genome-wide approach. However, these are yet to be applied in varicose vein populations.

**Genome-wide analysis**

The genome-wide association study (GWAS) approach is a new technology in which many (500,000–5,000,000) single-nucleotide polymorphism (SNP) markers are identified across the whole genome for an individual in a single analysis. Algorithms are applied that compare the frequencies of SNPs between disease and control cohorts. GWAS is rendered particularly powerful as even more variations in the genome can be imputed because of linkage disequilibrium. This is based on the principle that adjacent parts of the chromosome are inherited together with a disease-influencing allele. This then allows for unbiased exploration of the entire genome for any phenotype associations.

A particular advantage of the GWAS approach is its ability to identify novel markers and pathways that have not been previously considered to have a role in the condition of interest. For example, the chromosome 9p21 locus has been reproducibly associated with a number of arterial disease phenotypes.\(^{46,47}\) When this observation was first made it was not clear what the mechanism of action was, as the associated polymorphisms were not in the vicinity of any strong candidate genes. Subsequent analysis has revealed that this effect is due to an antisense non-coding RNA in the INK4 locus (ANRIL). Deletion of this region increases cardiovascular disease risk by altering the regulation of the cell cycle\(^{48}\) but the exact biological mechanism remains unclear.\(^{49}\) Without the GWAS approach it is unlikely that this association would have been identified.

It is also important to bear in mind the limitations of the GWAS approach. Very large numbers of subjects and controls are needed. While rare variants can be detected the enormous sample sizes required means that validated results are more typically found among more common alleles (>5–10%) of strong effect.\(^{50}\) A further problem stems from the very high false discovery rate associated with high levels of multiple gene testing. One approach to reducing the number of potential false associations is to perform an integrated pathway analysis. This involves using bioinformatic tools to compare information from gene expression and genome-wide studies to identify common biological pathways.\(^{51}\) This approach, however, like the candidate gene approach, may fail to identify unexpected polymorphisms, such as the previously mentioned ANRIL region in cardiovascular disease.

**Venous GWAS**

Clearly, we would suggest that the next step to help unravel genes implicated in venous disease is to consider a venous GWAS. Unfortunately, venous disease research has not leveraged this powerful tool in the same way that has occurred in other disease fields. For example, atherothrombotic disease has been examined in over a dozen GWASs completed to date\(^{52,53}\) while none have been completed so far for venous disease.

GWAS is likely to play an increasingly important role in validation of genetic polymorphisms.
identified to date and it may also help discover novel genes influencing in venous pathogenesis. These types of studies are not easy to accomplish and have significant pitfalls, and yet the application to varicose veins would be an important step forward.

A comprehensive protocol on how to design a genome-wide case-control association study was published in Nature. Based on this protocol a number of points need to be carefully considered prior to undertaking a venous disease GWAS:

- The venous disease phenotype needs to be clearly defined, including anatomy, severity and mode of assessment of varicosities. Any stratification of severity would be useful but this would result in a significant increase in sample size;
- Gender, time of onset and ethnicity need to be included in the analysis;
- A well-matched and screened control group needs to be identified, as prevalence of varicose veins in an unscreened control group may introduce bias;
- The studies need to be well powered, taking into account population stratification, linkage disequilibrium and multiple testing;
- Results need to be validated in an additional well-designed and powered cohort.

On balance, as long as a protocol is followed and both positive and negative associations are available for meta-analysis, venous GWAS may help unravel genes implicated in venous disease. In order to meet these design criteria, particularly with regard to sample size and replication, the collaboration of many groups of researchers is essential. To ensure that this happens, it is likely that an international consortium of researchers is necessary.

**Conclusion**

There is very little doubt that the development and progression of varicose veins and venous ulcer disease is multifactorial and includes a moderate-to-strong genetic component. The venous disease gene association studies conducted to date are few, with small sample sizes, and lack replication. Unfortunately, in population genetics such underpowered studies have been shown to be the source of a high level of false-positive association. Those wishing to extend our understanding of the genetic basis of venous disease will need to collaboratively design appropriate studies which will better distinguish true associations.

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