Title. Genetic polymorphisms of vein wall remodeling in chronic venous disease: a narrative and systematic review.

Running Title: Genetic polymorphisms in chronic venous disease.

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Abstract

Chronic venous disease encompasses a spectrum of disorders caused by an abnormal venous system. They include chronic venous insufficiency, varicose veins, lipodermatosclerosis, post-thrombotic syndrome, and venous ulceration. Some evidence suggests a genetic predisposition to chronic venous disease due to gene polymorphisms associated mainly with vein wall remodelling. The literature exploring these polymorphisms has not been reviewed and compiled thus far. In this narrative and systematic review, we present the current evidence available on the role of polymorphisms in genes involved in vein wall remodelling and other pathways as contributors to chronic venous disease. We searched the EMBASE, Medline, and PubMed databases from inception to 2013 for basic science or clinical studies relating to genetic associations in chronic venous disease, and obtained 38 relevant studies for this review. Important candidate genes/proteins include the matrix metalloproteinases (extracellular matrix degradation), vascular endothelial growth factors (angiogenesis and vessel wall integrity), FOXC2 (vascular development), HFE (venous ulceration and iron absorption) and various types of collagen (contributors to vein wall strength). The data on associations between these genes/proteins and the post-thrombotic syndrome is limited and additional studies are required. These associations might have future prognostic and therapeutic implications.
Introduction

Chronic venous disease (CVD) refers to a spectrum of overlapping diseases involving abnormalities of the venous system, both structural and functional. Chronic venous insufficiency (CVI) is a term that describes functional abnormalities of the venous system, but is often used to describe the full range of CVD manifestations such as varicose veins, venous ulceration, lipodermatosclerosis (LDS), and post-thrombotic syndrome (PTS) (essentially a secondary form of CVI).\(^1,2,3\) Almost a quarter of the adult population in the Western world has some form of CVD, but treatment is often delayed or deferred due to an underestimation of the prevalence and burden of the condition, leading to significant disability. Treatment is multifactorial, and often includes mechanical and/or surgical measures such as compression devices, leg elevation, ablations, and vein stripping.\(^1\)

CVI is a multifactorial disease, and although most commonly caused by valvular incompetence and venous hypertension, the exact pathogenesis remains unclear though various studies have suggested a potential genetic contribution.\(^4\) This condition may be primary (abnormalities of vein walls or valves), or secondary (after venous thrombosis; i.e. PTS). Risk factors include age, female gender, pregnancy, family history, obesity, and prolonged orthostasis. Clinical manifestations include leg pain, lower extremity edema, skin changes, varicose veins, and venous ulceration.\(^5\) Venous ulcers are a severe complication of CVI, though the underlying mechanisms are not fully known.

Varicose veins, a form of CVD and the most common manifestation of CVI, are caused by a loss of vessel wall homeostasis; venous hypertension leads to vein dilatation, distortion, leakage, and inflammation, causing valve and wall disease and reflux.\(^6,7,8\) Although sometimes caused by PTS, varicose veins are usually primary in nature. They are often hereditary, and result from extensive extracellular matrix (ECM) remodelling, leading to vein wall weakening or dysfunction.\(^9,10,11\)
PTS, a type of CVD, is an important and frequent chronic complication of deep venous thrombosis (DVT) that develops in 20-50% of patients (severe in 5-10%) following DVT despite appropriate anticoagulation. Risk factors include incomplete DVT symptom resolution, proximal or previous ipsilateral DVT, obesity, and increased age. Although the pathophysiology of PTS is not well understood, it involves venous hypertension caused by persistent venous obstruction and/or valvular reflux due to valve destruction, and recent evidence also supports a role for inflammation. Symptoms are similar to CVI and can be debilitating, often including constant or intermittent limb swelling, aching, cramps, or numbness/tingling (improved with rest or recumbency). Treatment involves symptomatic relief using graduated elastic compression stockings or compression devices, leg elevation, or a trial of horse chestnut seed extract as a last resort.

Recently, there has been some research into the genetic contributors and risk factors of CVD such as varicose veins and venous ulceration, mainly relating to certain noted polymorphisms in genes associated with vein wall remodelling. In fact, genetic risk factors are already known to affect wound progression and healing, and screening in this regard may aid in the planning of appropriate individualized treatment and prophylaxis. Also, various thrombophilic single nucleotide polymorphisms (SNPs) may contribute to CVI and ulcers by increasing the risk of DVT.

More recently, however, other SNPs have been studied in genes relating specifically to vein wall remodelling and pro-inflammatory or angiogenesis-regulating factors and receptors. While studies in this area do support a moderate to strong genetic predisposition toward CVI and varicose veins, the exact genes are still unknown – though they likely involve quantitative or qualitative defects in proteins associated with the vein wall, ECM, and cell organization/regulation. These polymorphisms, in turn, could plausibly increase the likelihood of CVD, including PTS after DVT, but evidence in this area is lacking.
Though various candidate genes have been identified as contributors to CVD, the evidence has not previously been compiled and critically reviewed. In this review, we use a systematic approach to explore the current evidence regarding genetic polymorphisms in vein wall structure and healing/remodelling as potential contributors to CVD, including PTS. Understanding these genetic associations may help clarify the underlying pathophysiology, identify patients at risk, and suggest novel therapeutic options.

Methods

In order to identify potential genes associated with CVD, we conducted a broad computerized literature search. We considered for inclusion any studies, either basic or clinical, describing or potentially describing an association between any gene, gene mutation or genetic polymorphism and any of the following conditions: varicose veins, chronic leg ulcers, chronic venous insufficiency, lipodermatosclerosis, post-thrombotic syndrome, or any combination of these. The search was conducted in September 2013 from the inception of each of the following databases: EMBASE, Medline (through the OVID interface), and PubMed. The search strategy used was: [“varicose veins” OR “chronic venous insufficiency” OR “leg ulcer” OR “post-thrombotic syndrome” OR “post thrombotic syndrome” OR “post-phlebitic syndrome” OR “post phlebitic syndrome”] AND [“genetics” OR “gene” OR “genes” OR “mutation” OR “polymorphism”]. We restricted the search to studies published in English. The retrieved references were initially screened for eligibility based on titles by one author and confirmed by another author. A preliminary list of potentially relevant studies was then independently screened by 2 authors based on titles and abstracts and a final list of studies to review in full was generated by consensus. Any studies whose relevance was unclear were reviewed in full. Quality of the studies was not assessed due to the lack of validated scales for the type of studies included. We did not plan a meta-analysis because we anticipated substantial heterogeneity among
studies. Our results are presented descriptively, by CVI subtype. Finally, although no standards exist for reporting systematic reviews of basic science studies, whenever possible we attempted to adhere to the available reporting standards.17,18

Results

Search results

The search process is summarized in Figure 1. The initial search generated 682 results. Initial screen by title alone resulted in 142 potentially relevant studies. The final selection for review in full included 41 studies of which 3 were excluded and 38 articles were included in the final review.

Genetic Polymorphisms and Chronic Venous Disease

Our literature search yielded various candidate genes that have evidence-based associations with a spectrum of CVD including CVI, varicose veins, LDS, venous ulceration, and PTS (Table 1). There was significant overlap in that these genes were often associated with more than one CVD manifestation (Figure 2), but they are presented here according to their most significant associations with a given subtype. The matrix metalloproteinases are discussed separately, given their consistent strong associations with various forms of CVD.

Varicose Veins

The ECM is a complex and dynamic framework of collagen, proteoglycans, elastin, glycoproteins, and cellular components. Degradation or destruction of the ECM disrupts the homeostasis of the vein (much of which is maintained by the matrix metalloproteinases – discussed later), thereby leading to varicosity. Vein wall weakness is a key player in the pathophysiology of varicose veins, and collagen is an important matrix component that provides strength; however, collagen dysregulation leads to vein wall abnormalities.4 In varicose veins, the total elastin content is decreased, type I collagen is upregulated, and type III collagen is downregulated.7,19 Jin et al.4 conducted a recent study that examined a 7-base pair insertion/deletion polymorphism (rs3917) in
the COL1A2 (α-2 type I collagen) gene. They found that this polymorphism upregulated COL1A2 expression and produced a 1.6-fold increase in CVI risk, and speculated that genetic variations in this gene alter transcriptional activity, affect mRNA structure, and ultimately allow expressional upregulation.4

Kowalewski et al.20 demonstrated increased expression of the cytokine VEGF-A and receptor VEGF R2 in the walls of varicose veins as compared to normal saphenous veins, particularly if complicated by thrombophlebitis. Similarly, Hollingsworth et al.21 observed increased transcription of VEGF and its receptors in varicose veins, reflecting a potential early role in varicogenesis. Of note, VEGF-A is involved in the maintenance of vessel wall integrity. Increased expression of VEGF-A or its receptor R2 leads to increased activation of nitric oxide synthase, which then leads to vessel wall damage mediated by oxygen free radicals, as well as decreased vessel tone that predisposes to venous stasis.20 VEGF also plays a key role in angiogenesis, so SNPs in the VEGF gene (C936T and -1780 T/C have been described) can be considered risk factors for impaired wound healing and venous ulceration.22

Chang et al.6 conducted a study using microarray bioinformatics that systematically explored various genes involved in biological pathways that may contribute to varicosity. The results showed that 32 genes were upregulated and 74 genes were downregulated in varicose veins, most of which were related to apoptosis and angiogenesis. Important examples of upregulated genes included: HSP90, ILK, and TGF-β1.6 In fact, Saito et al.23 earlier noted that TGF-β1 stimulates collagen synthesis and alters levels of matrix metalloproteinases (discussed later).

Mutations in the FOXC2 gene have also been associated with varicose veins. FOXC2 encodes a transcription factor involved in lymphatic and vascular development, and mutations in FOXC2 are seen in lymphoedema distichiasis, which is characterized by lymphoedema and varicose veins.7,24 Ng et al.24 conducted an early twin linkage study that strongly implicated FOXC2 in the development of varicose veins as a heritable condition. Similarly, knowing that
FOXC2 had previously been implicated in primary venous valve failure, Al-Batayneh et al.\textsuperscript{25} identified three specific SNPs in the FOXC2 gene that may contribute to varicose vein and hemorrhoid development. More recently, Mellor et al.\textsuperscript{26} conducted a small study of 18 patients and noted a strong association between FOXC2 and primary venous valve failure in both superficial and deep veins of the lower limb.

Other lesser known genes have also been examined in preliminary studies, and must be mentioned. Jeong et al.\textsuperscript{27} performed large scale mRNA screens among normal varicose veins, and found the greatest differential expression in the octamer-binding transcription factor-1 gene (Oct-1), which was upregulated in primary varicose veins. Cario-Toumaniantz et al.\textsuperscript{8} found overexpressed vitamin-K dependent matrix gla protein (MGP) in varicose veins, and speculated that its role in wall remodelling involved smooth muscle proliferation and mineralization processes.

**Venous Ulceration**

Grzela et al.\textsuperscript{22} reviewed 4 genes that may play a role in venous ulceration: TNF, FGF-R, estrogen receptor, and HFE. Levels of TNF\textsubscript{α} and interleukin-1 are higher in venous leg ulcers as compared to normal acute wounds, with certain SNPs (such as the -308A variant in TNF\textsubscript{α}) conferring a higher risk of venous ulceration compared to wild-type.\textsuperscript{5,22,28} Along with demonstrating the above-mentioned association with TNF\textsubscript{α}, Wallace et al.\textsuperscript{28} also identified a polymorphism in intron 10 of the BAT1 gene (HLA-B-associated transcript-1) as being a significant risk factor for venous ulceration.

Similarly, fibroblast growth factors (FGF) and their receptors (FGF-R) are cytokines that are imperative for the control of connective tissue regeneration in wound healing. Kowalewski et al.\textsuperscript{9} found increased acidic fibroblast growth factor (a-FGF) expression in the walls of varicose veins. They noted that these walls contained extensive ECM remodelling and altered collagen and glycosaminoglycan differentiation; a-FGF likely influences the expression of certain enzymes
through various pathways (ex. MAP kinase pathway) involved in ECM metabolism and varicosity. Furthermore, a-FGF synthesis is enhanced by hypoxia, which may be a consequence of venous stasis. Previous studies indicate that certain SNPs in fibroblast growth factor-receptor type 2 (FGF-R2), most frequently the polymorphism A2451G, are more commonly observed in CVI or ulceration. Though the mechanism is unclear, these SNPs likely result in lower expression of FGF-R2, causing impaired regeneration of connective tissue, longer vein wall re-epithelialization, and eventually CVI or ulceration. Nagy et al. conducted an association study and identified a SNP in the FGF-R2 gene that was more prevalent CVI patients with non-healing ulcers; as mentioned above, this likely led to abnormal re-epithelialization and angiogenesis.

Estrogen is a known contributor to ECM metabolism; in fact, hormone replacement therapy prevents CVI and topical estrogen promotes wound healing in the elderly by decreasing the inflammatory response. Though convincing associations have not been elucidated, several SNPs in the ER-β receptor have been associated with venous ulcers in the elderly. Ashworth et al. found that polymorphisms in the upstream regulatory regions of the ER-β gene were significantly associated with venous ulceration, but did not conduct further functional studies to determine the precise mechanism.

Hemochromatosis is an inherited iron overload disease caused by mutations in the HFE gene (most commonly allele C282Y), an MHC class 1-type membrane protein associated with β2-microglobulin. The mutated gene results in abnormalities in the regulation of iron absorption related to interactions between transferrin and its receptor. In patients with CVI, the HFE C282Y allele increases the risk of ulceration by almost 7-fold, likely due to accumulation of iron in tissues surrounding blood vessels, increased free radicals and oxidative stress, and upregulation of the inflammatory response which finally leads to tissue destruction. Moreover, Gemmati et al. found that the -8CG polymorphism in the FPN1 gene (ferroportin; involved in exporting iron out of the cell) increases susceptibility to leg ulcers.
Finally, Sam et al.\textsuperscript{32} suggested from preliminary data that mild to moderate hyperhomocysteinemia is common in patients with CVI (approximately 65%, especially with ulceration), and is associated in one-third of patients with an underlying MTHFR (methyleneetetrahydrofolate reductase) C677T homozygous polymorphism.

\textit{Lipodermatosclerosis}

LDS is a consequence of CVI, and is characterized by severe skin changes including hardening, atrophy, dark pigmentation, and edema. It often progresses to skin breakdown, venous ulceration, and delayed healing.\textsuperscript{33,34} deGiorgio-Miller et al.\textsuperscript{33} analyzed leg skin biopsies from patients with LDS and found enhanced cell proliferation and procollagen gene expression, as well as significant fibrotic changes which correlated directly with ulcer formation and healing time. Furthermore, imbalances in the matrix metalloproteinases and their inhibitors (discussed later) have also been implicated in LDS through the generation of epidermal and dermal skin defects.\textsuperscript{34}

\textit{Post-Thrombotic Syndrome}

PTS is a frequent yet poorly understood complication of DVT. Previous studies have explored genes involved in vein wall remodelling in relation to CVI and its manifestations (ex. varicose veins and venous ulcers). These are also manifestations of PTS, and it is logical to infer that polymorphisms in these very genes may potentially increase the risk of PTS as well. In fact, Deatrick et al.\textsuperscript{35} noted ongoing vein wall remodelling 6 months after an acute DVT that was associated with biomarkers such as MMP-9 that directly correlate with resolution and predict PTS. There is, however, a dearth of literature that directly associates the above genes with PTS.

An early study by Dahi et al.\textsuperscript{36} demonstrated increased MMP-2 and MT1-MMP activity (potentially mediated by thrombin) during DVT resolution, which, in turn, increased the risk of PTS. Wojcik et al.\textsuperscript{37} used a mouse model to conclude that IL-6 (interleukin-6) is associated with reduced monocyte recruitment, leading to reduced vein wall thickness and fibrosis. Given that PTS involves extensive perivenous and mural fibrosis, IL-6 may serve as a therapeutic target to prevent
these fibrotic complications. Another recent mouse model study by Deatrick et al.\textsuperscript{38} reveals that MMP-9 modulates vein wall collagen content and contributes to inflammation and fibrosis, thus implicating it as a potential target to reduce the fibrotic complications of PTS.

To our knowledge, there has been no study to date that has analyzed polymorphisms in the above-mentioned candidate genes to uncover a direct association with PTS. Some of the studies presented in Table 1 involve tissue blocks from veins complicated by thrombophlebitis, and some of the patients were noted to have a history of venous thrombosis (however, scant patient data was provided), but none of the studies specifically included PTS as a subset.

\textit{The Matrix Metalloproteinases}

Perhaps the most studied genes in venous disease are the matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP). A systematic review by Lim \textit{et al.}\textsuperscript{7} revealed that MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, TIMP-1, and TIMP-3 were all upregulated in varicose veins. Similarly, Raffetto \textit{et al.}\textsuperscript{39} noted that MMPs are involved in ECM degradation, which subsequently leads to venous remodelling, structural wall changes, and ultimately venous dilatation and valve dysfunction; they are thus heavily involved in the pathogenesis of CVD.

Though the molecular mechanisms of these genetic anomalies have not been fully elucidated, it appears that MMPs regulate or degrade the ECM through hydrolysis, while TIMPs are tissue inhibitors of MMPs that influence vascular remodelling. Hence, an imbalance between these proteins may lead to abnormalities in the vessel wall and, ultimately, vascular disease such as varicosity and ulceration.\textsuperscript{7,35,40} Moreover, misregulation of MMP activity and TIMP counter-regulation contributes to impaired ulcer healing.\textsuperscript{34} An early study by Saito \textit{et al.}\textsuperscript{23} suggested that increased MMP-2 levels affect tissue remodelling and contribute to a pro-ulcer environment. Subsequently, Herouy \textit{et al.}\textsuperscript{41} examined stasis dermatitis, a common consequence of impaired venous drainage characterized by dermal neovascularization, and noted elevated MMP-1, -2, -13 and diminished TIMP-1, -2 in the skin lesions. More recently, Xu \textit{et al.}\textsuperscript{40} showed that specific
polymorphisms in the MMP-9 and TIMP-2 genes potentially put patients at a higher risk for developing varicose veins. Deatrick et al. examined vein remodelling and associated gene expressions, but did not correlate results with the development and severity of PTS. However, their preliminary data did show increased MMP-9 and decreased TLR-9 expression in acute DVT.

MMP-9 levels have been shown to be increased in varicose veins and venous ulcers, and some data suggests a potential role in DVT resolution, with gene deletions being associated with less collagen deposition. In fact, Beidler et al. used multiplexed protein analysis to show that all MMPs except MMP-7 were highly expressed in venous leg ulcers, especially MMP-8 and MMP-9. In addition, Singh et al. analyzed various SNPs associated with venous leg ulcers and found that MMP-12 gene polymorphisms may have a role in ulcer progression.

Interestingly, Kurzawski et al. demonstrated that polymorphisms in MMP-1 and MMP-3 did not predict susceptibility to varicose veins, but this study likely did not have the power to uncover the milder effects of these genes on an otherwise multifactorial disorder.

**Discussion**

In this review, we examined different candidate genes and their polymorphisms (mostly SNPs) that have been linked to various forms of CVD.

In assessing the above studies, a few candidate genes should be highlighted as having the strongest link to the development of CVD (as we could not perform a meta-analysis, these assessments are qualitative, based on the available evidence). Three studies agree that SNPs in FOXC2, a transcription factor involved in lymphatic and vascular development, provide a strong link toward venous varicosity, valve failure, and hemorrhoids. On the other hand, a polymorphism in the HFE gene increases the risk of venous ulceration by almost 7-fold, while also causing the known dysregulation in iron absorption. The MMPs (and, to a lesser extent, their inhibitors – TIMPs) are perhaps the best studied in venous disease. MMPs are involved in ECM degradation...
and structural vein wall changes, ultimately contributing to venous remodelling, dilatation and valve dysfunction. They are thus heavily involved in the pathogenesis of CVD. The exact mechanisms have yet to be elucidated and further research is required in this area.

Although genetic markers in CVD have been examined in some detail, there is a lack of evidence correlating these genes with the development and severity of PTS. If these polymorphisms are potentially associated with CVI, LDS, varicose veins, venous ulcers, and DVT resolution, it is plausible that they could also provide an underlying substrate for the development of PTS. The MMPs have once again been implicated in this respect, but data is scarce and further research is warranted.

In general, a limitation of all of the studies included in this review relates to the fact that they were conducted either in animal models, in very limited numbers of patients, or using surgical samples (e.g. saphenectomy). It is very difficult to assess the quality of each study since no standardized tools exist in this regard, in contrast to the quality assessment scales used for clinical studies. We believe that the included studies were methodologically sound; however, we cannot totally rule out the possibility of biased results.

Clearly, the genetic predisposition to CVD, particularly PTS, is controversial and unclear at best, and more studies are needed to clarify these associations. Earlier studies have promoted the use of a genome-wide approach; this allows for the identification of previously unknown markers, and may be a consideration for the future. Clinically, this would be a novel way to re-examine a patient’s propensity toward developing CVD, predict those who go on to develop it, and provide a better understanding of the underlying mechanisms to ultimately improve treatment options.

Conclusions

Herein, we examine the role of various candidate genes and their polymorphisms in the development of CVD, including the lesser studied PTS. Our observations support a genetic
predisposition to CVD related to vein wall remodelling. Genes of significance include FOXC2, HFE, and the MMPs, all of which show strong associations with varicose veins, CVI, or venous ulceration. The data surrounding PTS is more limited, but given that it is a type of CVD with similar manifestations, we may infer that some of the above genes may be implicated. Thus, further studies are needed to examine these associations more directly. Most importantly, given the burden of this disease worldwide and the paucity of treatment options currently available, studying these polymorphisms could potentially allow us to better identify patients at higher risk of developing CVD, and also provide novel therapeutic targets.

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**Authorship Contributions**

ALL and SK conceived the idea. VB and ALL performed the systematic review. VB wrote an initial draft of the article. VB, SK, and ALL reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

**Conflict of Interest Disclosures**

The authors declare no competing financial interests in relation to this work.
References


Table 1: Candidate gene polymorphisms/abnormalities and their proposed evidence-based associations in chronic venous disease.

<table>
<thead>
<tr>
<th>AUTHOR (YEAR)</th>
<th>STUDY DETAILS</th>
<th>GENE</th>
<th>PROPOSED FUNCTION</th>
<th>POLYMORPHISMS / ABNORMALITY</th>
<th>RESULTS</th>
<th>STRENGTH OF ASSOCIATION</th>
</tr>
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<tbody>
<tr>
<td>Kowalewski (2011)&lt;sup&gt;20&lt;/sup&gt;</td>
<td>basic science</td>
<td>VEGF-A / VEGF-R2</td>
<td>Vessel wall integrity, angiogenesis</td>
<td>Increased mRNA / protein expression</td>
<td>Increased VEGF-A &amp; VEGF-R2 content in VV vs. normal wall tissue VEGF-A: 41.76 vs. 25.79 ng/g VEGF-R2: 57.47 vs. 28.92 ng/g</td>
<td>P &lt; 0.001</td>
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<tr>
<td>Chang (2009)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>basic science / genetic microarrays</td>
<td>HSP-90</td>
<td>Protein degradation</td>
<td>Upregulated</td>
<td>32/74 genes upregulated in VV compared to control</td>
<td>VV:CV (control veins) ≥ 2-fold increase in intensity</td>
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<td></td>
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<td>ILK</td>
<td>Cell signalling, apoptosis</td>
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<td></td>
<td>TGF-β1</td>
<td>Cell proliferation and apoptosis</td>
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<tr>
<td>Ng (2005)&lt;sup&gt;24&lt;/sup&gt;</td>
<td>basic science / twin linkage analysis using marker gene D16S520</td>
<td>FOXC2</td>
<td>Lymphatic and vascular / venous development</td>
<td>Functional variants</td>
<td>Concordance rates significantly higher for monozygotic vs. dizygotic (VV: 67% vs. 45%)</td>
<td>P = 2.2E-6</td>
</tr>
<tr>
<td>Mellor (2007)&lt;sup&gt;26&lt;/sup&gt;</td>
<td>basic science</td>
<td>FOXC2</td>
<td>FOXC2 mutations (varied)</td>
<td></td>
<td>Great saphenous vein reflux in 18/18 with various FOXC2 mutations (deep vein reflux: 14/18) vs. 1/12 in referents</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Al-Batayneh (2008)&lt;sup&gt;25&lt;/sup&gt;</td>
<td>basic science / genomic analysis and sequencing</td>
<td></td>
<td></td>
<td></td>
<td>SNPs in proximal upstream region of FOXC2 in VV / hemorrhoid patients (not in controls) --SNP 1: 5/24 (20.8%) --SNP 2: 2/24 (8%) --SNP 3: 1/24 (4.2%)</td>
<td>N/A</td>
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<tr>
<td>Study</td>
<td>Type of Study</td>
<td>Gene / Gene expression / Protein expression</td>
<td>Findings</td>
<td>P-Value</td>
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<tr>
<td>Sansilvestri (1998)(^1)</td>
<td>basic science / vein immuno-staining</td>
<td>Type I / III collagen, ECM integrity</td>
<td>Type III collagen deposition decreased</td>
<td>P &lt; 0.005</td>
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</table>
| Cario-Toumani-antz (2007)\(^2\) | basic science / gene expression    | Type I collagen, Vein wall remodelling      | Relative mRNA expression in varicose vs. nonvaricose vein tissue: Type I collagen 2.33, Type III collagen 1.70 | Type I: P < 0.001  
|                                |                                    |                                             |                                                                           | Type III: P < 0.01 |
| Jeong (2008)\(^2\)           | basic science / gene expression    | Oct-1, Cellular response to stress / regulation of gene expression | Upregulation                                                              | P < 0.001 |
| Wallace (2006)\(^2\)         | basic science case-control study   | TNFα, Inflammation                         | Presence of allele: 43.1% in ulcer patients vs. 22.6% in controls         | OR: 2.48  
P = 0.000155 |
|                                |                                    | BAT1, Apoptosis; linkage with TNFα         | Presence of allele: 28.8% in ulcer patients vs. 16.3% in controls         | OR: 2.00  
P = 0.012 |
| Kowalewski (2009)\(^3\)      | basic science / gene expression    | a-FGF, FGF-R2                               | Vein wall aFGF content: 32.21 pg/g of protein in VV vs. 24.68 pg/g in normal veins  
|                                |                                    | Connective tissue regeneration, wound healing, ECM metabolism | No significant difference in FGF-R expression | P < 0.001 |
| Nagy (2004)\(^4\)           | basic science / genetic analysis   | FGF-R2: 2451A → G, [Grzela et al.] (rs7895676) (rs2981578) | Heterozygotes: 53.66% in leg ulcer patients vs. 45.12% in controls  
|                                |                                    |                                             | Homozygotes: 23.17% vs. 13.42%                                           | P = 0.0103 |
| Ashworth (2008)\(^5\)        | basic science case-control study   | ERβ, Estrogen receptor; inflammatory and repair processes | Upstream regulatory regions, including 0N exon and promoter  
|                                |                                    |                                             | Cases vs. controls  
|                                |                                    |                                             | 1. 58% vs. 49%  
|                                |                                    |                                             | 2. 34% vs. 25%  
|                                |                                    |                                             | 3. 13% vs. 7%  
|                                |                                    |                                             | 4. 47% vs. 38%  
|                                |                                    |                                             | 1. P = 0.025  
|                                |                                    |                                             | 2. P = 0.010  
|                                |                                    |                                             | 3. P = 0.002  
<p>|                                |                                    |                                             | 4. P = 0.013 |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Methodology</th>
<th>Gene/Protein</th>
<th>Homology/Function</th>
<th>Association</th>
<th>OR/Significance</th>
</tr>
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<tbody>
<tr>
<td>Gemmati (2009)³¹</td>
<td>basic science / DNA-array study</td>
<td>HFE</td>
<td>Regulation of iron absorption</td>
<td>T-T-T-A haplotype significantly associated with venous ulceration</td>
<td>2.9</td>
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<td></td>
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<td></td>
<td>282G → A</td>
<td>Heterozygotes: 9.9% in ulcer patients vs. 1.8% in controls Homozygotes: 0.6% vs. 0%</td>
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<tr>
<td></td>
<td></td>
<td>FPN1</td>
<td>Iron export</td>
<td>Heterozygotes: 34% in ulcer patients vs. 30.8% in controls Homozygotes: 8.6% vs. 2.3%</td>
<td>5.2 P = 0.005</td>
</tr>
<tr>
<td>Sam (2003)³²</td>
<td>basic science / genetics</td>
<td>MTHFR</td>
<td>Homocysteine metabolism</td>
<td>Based on CEAP classification: C2-3: 10% homozygous for C677T C4-6: 20% Overall, 15% homozygous for C677T vs. 5% in healthy popln</td>
<td></td>
</tr>
<tr>
<td>Jin (2013)⁴</td>
<td>basic science / DNA extraction and genotyping</td>
<td>α-2 Type I collagen (COL1A2)</td>
<td>ECM integrity</td>
<td>rs3917 (7-base pair indel polymorphism in 3’UTR)</td>
<td>Indel polymorphism present in 12.2% of CVI cases vs. 8% controls</td>
</tr>
<tr>
<td>deGiorgio-Miller (2004)³³</td>
<td>basic science / pathology + gene expression</td>
<td>Pro-collagen (COL1A1)</td>
<td>ECM integrity</td>
<td>Overexpression</td>
<td>Increased procollagen type I mRNA (COL1A1) in LDS vs. other patient groups due to increased mesenchymal cells / fibroblasts [LDS vs. control: 180 vs. 97 per 50000 μm²]</td>
</tr>
<tr>
<td>Gemmati (2009)³¹</td>
<td>basic science / DNA-array study</td>
<td>MMPs</td>
<td>ECM degradation</td>
<td>MMP-12: -82A → G Smaller ulcer size in patients with -82GG genotype -82GG: 5.4 ± 2 cm² -82AA + AG: 11.1 ± 17.1 cm²</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>Xu (2011)⁴⁰</td>
<td>basic science / genotyping</td>
<td>MMPs</td>
<td>ECM degradation</td>
<td>MMP-9: -1562C/C -1562C allele frequency: 81.7% in VV group vs. 48.3% in controls</td>
<td>P = 0.000 OR: 6.102</td>
</tr>
<tr>
<td>Deatrick (2013)³⁸</td>
<td>basic science [mice]</td>
<td>MMPs</td>
<td>ECM degradation</td>
<td>MMP-9 -/- Vein walls of MMP9 -/- mice had 45% less collagen content / fibrosis vs. controls at 8 and 21 days after stasis thrombosis injury</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>
| Saito (2001)²³ | basic science / gene expression | Overexpression of MMP-1 mRNA and MMP-2 protein | CVI patients, class 1-6  
MMP-1 mRNA: 0.002 (control) vs. 4.15 (class 4) vs. 31.2 (class 6)  
TIMP-1: 2.56 (control) vs. 23.45 (class 6)  
Active component of MMP-2 increased in class 4-5 patients relative to MMP-1 and TIMP-1 | MMP-1 mRNA: P < 0.01  
TIMP-1: P < 0.05 |
<table>
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<tbody>
<tr>
<td>Herouy (2001)⁴¹</td>
<td>basic science / gene expression</td>
<td>Overexpression of MMP-1,2,13</td>
<td>Increased expression of MMP-1, -2 and -13 mRNA in lesional skin (stasis dermatitis) vs. controls</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Beidler (2008)⁴²</td>
<td>basic science / gene expression</td>
<td>Overexpression of MMP-1,2,3,8,9,12,13</td>
<td>MMP-1,2,3,8,9,12,13 protein levels elevated in ulcer tissue vs. controls; MMP-1,9,8,13 had at least 80-fold increase</td>
<td>P &lt; 0.005 (majority)</td>
</tr>
<tr>
<td>Dahi (2005)³⁶</td>
<td>basic science / gene expression [mice]</td>
<td>Overexpression of MMP-2 and MMP-14 (MT1-MMP)</td>
<td>During resolution of DVT: 71% increase in MMP-2 activity in ligated cavae (DVT generated) vs. controls; MMP-14 mRNA upregulated 2.5-fold compared to controls</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Cario-Toumani-antz (2007)⁸</td>
<td>basic science / gene expression</td>
<td>Overexpression of TIMP-1</td>
<td>Higher relative TIMP-1 mRNA expression in varicose vs. nonvaricose veins (2.06 ± 0.26)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>
| Xu (2011)⁴⁰ | basic science / genotyping | TIMPs Inhibitors of MMP | TIMP-2: -418G → C | Allele frequency: 23.3% in VV group vs. 14.2% in controls  
P = 0.038  
OR: 2.213  
Unclear significance |
| Herouy (2004)³⁴ | basic science / gene expression | Diminished TIMP-1,2 | Low gene expression and immunoreactivity of TIMP-1,2 in stasis dermatitis vs. controls | Not significant |
| Wojcik (2011)³⁷ | basic science [mice] | IL-6 Inflammation | IL-6 as therapeutic target | Mice with IVC thrombus treated with anti-IL6 had decreased vein wall fibrosis (intimal thickness reduced by 44%) vs. those treated with control rat IgG | P < 0.05 |

**Abbreviations:** CVI, chronic venous insufficiency; LDS, lipodermatosclerosis; VV, varicose veins; PTS, post-thrombotic syndrome
Figure Legends

Figure 1: Flow diagram of the systematic review

Figure 2: Candidate genes and their overlapping associations with the spectrum of chronic venous disease

Figure 2 footnote: See text for definitions
682 results identified

Preliminary screen by title

142 results screened by title and abstract (in consensus with 2nd reviewer)

41 full-text articles assessed for eligibility

38 studies included in qualitative synthesis (meta-analysis not performed)

540 results excluded (not related to conditions of interest)

101 results excluded (not related to conditions of interest)

3 full-text articles excluded due to irrelevance, insufficient evidence, and/or unknown conclusions
Figure 2

Chronic Venous Insufficiency / Venous Ulcers

Varicose Veins

COL1A2
FOXC2
HSP90
ILK
MGP
Oct-1
TGF-β1
Type 1 & III collagen
VEGF -A
VEGF-R

a-FGF
FGF-R
BAT1
ER-β
FPN1
HFE
IL-1
MTHFR
Procollagen
TNF-α
VEGF

MMPs/TIMPs
COL1A2
Procollagen

Lipodermatosclerosis

Procollagen

MMPs
IL-6
TLR-4
TLR-9
SELPG

Post-thrombotic Syndrome
Genetic polymorphisms of vein wall remodeling in chronic venous disease: a narrative and systematic review

Vighnesh Bharath, Susan R. Kahn and Alejandro Lazo-Langner