

Perspective

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# The genetic constituent of varicose vein pathogenesis as a key for future treatment option development

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**How to cite this article:** Smetanina MA, Shevela AI, Gavrilov KA, Filipenko ML. The genetic constituent of varicose vein pathogenesis as a key for future treatment option development. *Vessel Plus* 2021;5:19. <https://dx.doi.org/10.20517/2574-1209.2021.17>

**Received:** 31 Jan 2021 **First Decision:** 1 Mar 2021 **Revised:** 15 Mar 2021 **Accepted:** 24 Mar 2021 **Available online:** 17 Apr 2021

**Academic Editor:** Alexander D. Verin **Copy Editor:** Yue-Yue Zhang **Production Editor:** Yue-Yue Zhang

## Abstract

This perspective focuses primarily on the fundamental part of phlebology, where most attention is paid to the genetic aspects of the pathogenesis of varicose vein disease and where the main breakthrough advances in this area of research are discussed. We propose a direction for further actions to replenish molecular genetic knowledge about the main drivers of pathological processes in varicose vein disease and to complete the creation of an entire picture of pathogenesis, which, in turn, may serve as a key for the development of treatment options in the future in order to translate research evidence into clinical practice.

**Keywords:** Varicose veins, pathogenesis, genetics, epigenetics, targets

Varicose vein disease (VVD)-the main nosological form of chronic venous diseases-is a multifactorial disease. Risk factors for developing venous pathologies include genetic factors and environmental factors. The risk factors at their basis should be attributed to: (1) predisposing; and/or (2) affecting factors. Genetic, as well as epigenetic, factors seem to be part of both (1) and (2): the mechanical effect leads to a cascade of



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physiological processes, and existing genetic factors can aggravate the clinical situation, and vice versa. However, unfortunately, the genetic factors that are primarily involved in pathological processes are not yet fully understood, and we still have to understand how to formalize the genetic contribution to the phenotype of a person with varicose veins (VVs). The role of genetics in this context can hardly be overestimated, since genetics is one of the constituent parts, in general, of the nucleation of a protein from DNA, where both biochemical and molecular biological processes are involved (i.e., replication, transcription and translation, from what and how protein is synthesized). Moreover, epigenetics can play an equally important role.

## HEREDITARY CONSTITUENT OF VARICOSE VEIN PATHOGENESIS-AN OVERVIEW

The first mention of VVs in the PubMed database dates back to the early 19th century. In the first half of the 20th century, one can find explanations of the hereditary nature of the disease, the most judicious of which was given by the British scientist Ottley<sup>[1]</sup>. Having presented a detailed analysis of a series of fifty cases of VV treatment, the author concluded that inheritance occurs according to the dominant type (even taking into account the cases of the “missed generation” - the absence of manifestation of the disease in one of the generations), because, in general, there was a higher percentage of sufferers than with a recessive defect. In 1949, for example, as a result of histological studies, American scientists Wagner and Herbut drew conclusions about the hereditary factor of VVD; in addition, they were inclined to believe that dilatation of the vein wall precedes valvular insufficiency, which is usually purely functional and secondary<sup>[2]</sup>, which was subsequently supported by other scientists<sup>[3,4]</sup>. The significance of genetic factors in the etiology of VVD was also emphasized in the works of the Danish and Swedish scientists Hauge and Gundersen, who, based on the information they collected about the parents and siblings of 250 patients, concluded that the determination of the disease by one gene is unlikely, and inheritance seems to be multifactorial<sup>[5]</sup>. In 1974, Czechoslovakian scientists Matousek and Prerovsky estimated the heritability of primary varicose veins is up to 50% upon assuming the hypothesis of polygenic inheritance<sup>[6]</sup>. Later on, a prominent role of heredity in the development of VVs was demonstrated by the French scientists Cornu-Thenard *et al.*<sup>[7]</sup>. However, due to the fact that their study did not include examination of siblings or the third generation, they were unable to determine the genetic model of inheritance, and therefore they doubted autosomal dominant and autosomal recessive inheritance models, citing possible “pros” and “cons”. A little later, in 1998, Chinese scientists Guo and Guo, based on the results of the genetic analysis of VVD, concluded that in most cases this disease is compatible with an autosomal dominant inheritance of incomplete penetrance, while some of the cases were sporadic, which prompted them to assume a recessive model possible as well<sup>[8]</sup>. Indeed, with an autosomal dominant type of inheritance: (1) the disease is transmitted vertically and is diagnosed in each generation, but, due to incomplete penetrance in the transmission of a trait, sometimes one generation is skipped; (2) phenotypically “normal” family members do not transmit the disease to their offspring; and (3) both men and women can inherit the disease with equal frequency. After analyzing the literature data<sup>[9]</sup>, we tend to believe that the inheritance pattern of VVD is autosomal dominant with incomplete penetrance.

Subsequently, inheritance patterns and the results of family and twin studies were examined in 2003 by the French scientist Pistorius<sup>[10]</sup>, who hypothesized about the possible genetic heterogeneity of VVD, suggesting the existence of different genotypic profiles with a similar phenotype. Many studies by other scientists have also shown a significant genetic contribution to the etiology of VVD, as reported in a review by the New Zealand group of Krysa *et al.*<sup>[11]</sup>. Since then, genetic research has continued and gained strength and momentum. In recent decades, a lot of genetic research, particularly associative research, has been carried out.

## GENETIC ASSOCIATION STUDIES ON VARICOSE VEIN PATHOGENESIS-AN OVERVIEW

At the initial stages of the interaction of genetics and phlebology, associative studies of the “case-control” type were carried out, i.e., when the hypothesis is tested whether a genetic marker is found more often in a group of patients than in a control group. Originally, a gene-candidate approach was used, i.e., when one gene or a group of genes was selected, which can participate in the manifestation of some trait with a high probability. These genes were studied using genetic methods known at that time, and many of the studies reviewed provide conflicting data on the clinical significance of genetic associations. In particular, there are several distorting factors that make it difficult to compare between studies. There are different criteria for inclusion and exclusion, subjectivity in diagnosis, the presence of comorbidities, different sources of control groups, size disparity between the groups and differences between ethnic groups. Our 2016 review “The genetic base of chronic venous disease: a review of modern concepts”, which is highly scrutinizing from a genetic point of view, provides the detailed information available at that time about the relationship of genetic factors with the development of chronic venous disease<sup>[12]</sup>. Thus, the table “Polymorphic variants/mutations of candidate genes and their associations with chronic venous disease” shows not only the gene, the type of mutation/single nucleotide polymorphism (SNP) and the reference to the article, but also the sample size and the degree of this association, which is decisive for the significance of this association. In the studies performed in our laboratory, it has been shown that, for example, for polymorphic variants of genes *AGGF1* (rs13155212, rs7704267)<sup>[13]</sup>, *MTHFR* (rs1801133) and *MTR* (rs1805087)<sup>[14]</sup>, no associations with the risk of VVD in ethnic Russians were found. There were also no associations found for regulatory SNPs of matrix metalloproteinase genes *MMP1* (rs1799750), *MMP2* (rs243865), *MMP3* (rs3025058) and *MMP7* (rs11568818)<sup>[15]</sup>, whereas the rare rs1800562 A allele in the *HFE* gene leading to the accumulation of iron in the patient’s tissues<sup>[16]</sup> and polymorphic variants rs1035550 C>T and rs34221221 T>C of the transcription factor *FOXC2* gene<sup>[17]</sup> were associated with an increased risk of VVD. However, none of these associations reached statistical significance after adjustment for multiple comparisons. For the polymorphic locus rs2010963 G > C in the *VEGFA* gene for the vascular endothelial growth factor, an association with a decreased risk of VVD was revealed, and its significance remained after applying the Bonferroni correction<sup>[18]</sup>. On the sample of ethnic Russians, we also revealed the association of functional SNP rs1024611 in the regulatory region of the *MCP1* gene with the increased risk for primary VV development, which was prominent in C2-class patients, in patients younger than 30 years old at disease onset and in patients with negative anamnesis<sup>[19]</sup>. Thus, there was other evidence for an inflammatory component implication in the pathogenesis of VVs. Later on, we applied a candidate-gene approach to test the implication of 13 functional polymorphisms in the inflammation-related genes to VVD and revealed the association of C allele of the *IL6* rs1800795 and the reverse association of T allele of the *HIF1A* rs11549465, ATTG deletion of the *NFKB1* rs28362491 and A allele of the *TNF* rs3093661 with an increased risk of primary VVs<sup>[20]</sup>. Notwithstanding, the statistical significance level turned insignificant after correction for multiple comparisons. However, that study had some limitations; therefore, not all associations could be false discoveries.

Entering the era of opportunities and thorough screening of the genome, methylome, transcriptome, proteome, etc., we obtain new unprecedented potential for research on the molecular pathology of venous diseases using agnostic approaches (i.e., free from the original hypothesis) without the need to focus on individual genes. Figuratively, the candidate gene and omics approaches for research can be compared with lighting a street with one lantern or many lanterns, respectively. Obviously, in the latter case, it becomes much easier to find something on it.

One of the biggest genome-wide association studies (GWASs) on VVs was performed by the American 23andMe company scientists who identified a group of 12 SNPs and genes corresponding to them: rs507666

(*ABO*), rs966562 (*XKR5*, *ANGPT2*, *AGPAT5*), rs7111987 (*ADM*, *AMPD3*), rs11121615 (*CASZ1*), rs111434909 (*ANGPT1*), rs145218303 (*ARGHAP6*), rs6905288 (*VEGFA*), rs4516218 (*PIEZO1*), rs4463578 (*ZIC3*, *FGF13*), rs6062618 (*SOX18*, *TCEA2*), rs6712038 (*PPP3R1*, *CNRIP1*) and rs79607156 (*THEG5*, *ZNF507*)<sup>[21]</sup>. However, those associations were not replicated. Another genome-wide association analysis (combining the discovery and replication stages) for chronic venous disease performed by a German group revealed robust associations within the two loci and genes corresponding to them, namely rs17278665 (*EFEMP1*) and rs727139 (*KCNH8*), and suggestive association within rs2030136 (*SKAP2*)<sup>[22]</sup>. Noteworthy, we found the *EFEMP1* gene is upregulated in VVs compared to non-VVs<sup>[23]</sup>. The first large-scale genetic association study for primary varicose veins performed in our laboratory on the Russian population using exome genotyping identified a promising association signal at chromosome 6 within classical major histocompatibility complex class III subregion, with the most statistically significant association being shown in a combined analysis (discovery and replication stages) for polymorphism rs4151657 in the *CFB* (complement factor B) gene<sup>[24]</sup>, which points to immune system involvement in VV pathogenesis.

Quite a few works have been done to replicate top associations from GWASs for VVD using independent datasets. One of them, performed in our laboratory, aimed to verify the associations revealed by Bell *et al.*<sup>[21]</sup> and Ellinghaus *et al.*<sup>[22]</sup> using two independent groups of patients: (1) ethnic Russian individuals; and (2) genetic data on a large population-based cohort of British residents obtained from UK Biobank. We also aimed to perform a meta-analysis<sup>[25]</sup>. After combining the original GWAS results and replication studies by a meta-analysis, the following polymorphisms passed a genome-wide significant threshold: rs11121615, rs6712038, rs507666, rs966562, rs7111987, rs6062618, rs6905288, rs111434909, rs4463578, rs111434909 and rs4463578. Most of them are located near or within the genes involved in vascular development, remodeling and inflammation, which implicates these processes in VV pathogenesis. None of those SNPs were from the study of Ellinghaus *et al.*<sup>[22]</sup>, and the only one that reached a nominal significance level ( $P < 0.05$ ) in the Russian cohort was rs11121615 (*CASZ1*). Thus, the set of polymorphic variants of genes is indeed not the same in different populations.

Recently, two fairly large bioinformatics (non-experimental) works using modern approaches to big data analysis were published almost in parallel by American and Swedish scientists in 2018 in *Circulation*<sup>[26]</sup> and Russian scientists in 2019 in *PLoS Genetics*<sup>[27]</sup>. After analyzing data from the UK Biobank, the authors of these papers presented a comprehensive genetic and epidemiological study on VVs and identified new clinical and genetic risk factors. Fukaya *et al.*<sup>[26]</sup> demonstrated that greater height has a causal role in varicose vein development and discovered a strong genetic correlation between varicose veins and deep vein thrombosis (which, in our opinion, could be because some VVs in their study were not primary since VVD and DVT directly and inversely correlate with each other). Their large-scale GWA of VVs among 337,536 individuals (9577 cases and 327,959 controls) identified the top 30 genetic loci: rs11121615, rs2911463, rs2861819, rs28558138, rs8053350, rs3101725, rs11135046, rs7773004, rs12625547, rs236597, rs7614922, rs73107980, rs7469817, rs2241173, rs816943, rs1061539, rs1549063, rs16828263, rs9719461, rs2263321, rs247749, rs75522736, rs553399706, rs62512472, rs584768, not available SNP on chr6 position 127452639 (build37), rs2089657, rs12594708, rs186005582 and rs192647746. They denoted the genes in closest proximity to the variants (and not only those genes): *CASZ1*, *LBH*, *PPP3R1*, *IGSF11*, *GATA2-AS1*, *STIM2*, *TEMN3-AS1*, *AGGF1*, *EBF1*, *SLC12A2*, *HISTIH3G*, *HCG9*, *HLA-B*, *LINC02549*, *RSPO3*, *ALDH8A1*, *PRKAR1B*, *CNGB3*, *DEC1*, *HDAC7*, *DAOA*, *LINC00924*, *GLG1*, *CTU2*, *PIEZO1*, *KCNJ2*, *LINC01152*, *MAMSTR*, *NFATC2*, *ZNF512B*<sup>[26]</sup>. Shadrina *et al.*<sup>[27]</sup> used genetic association data on VVs for 337,199 individuals (6958 cases and 330,241 controls) and demonstrated direct causal effects of the anthropometric traits, such as height and weight (and not only those traits), as well as plasma levels of immune-related proteins MICB and CD209. Their large-scale GWA identified 12 reliably associated loci that explain 13% of

the SNP-based heritability of VVs, prioritizing the most likely causal genes (shown in brackets): rs11121615 (*CASZ1*), rs2911463 (*PIEZO1*, *CTU2*), rs2861819 (*PPP3R1*, *PNO1*), rs3101725 (*SLC12A2*, *FBN2*, *LINC01184*), rs11135046 (*EBF1*), rs28558138 (*STIM2*), rs7773004 (*HFE*), rs12625547 (*NFATC2*), rs2241173 [*SOX9*, *AC005152.3* (*LOC102723505*)], rs73107980 [*COL2A1*, *RAPGEF3* (*EPAC1*)], rs9880192 (*GATA2*) and rs236530 (*KCNJ16*, *KCNJ2*)<sup>[27]</sup>. All of those SNPs are the same as those found by Fukaya *et al.*<sup>[26]</sup>, and only the last two are not in the top 30. Thus, the genetic risk factors found in both works broaden the understanding of VV pathogenesis and can help in further studies to elucidate the mechanisms of the development of the disease.

## GENE EXPRESSION STUDIES ON VARICOSE VEIN PATHOGENESIS-AN OVERVIEW

In addition to genomic studies, one of the ways to “decipher” the possible stages of VV pathogenesis is to study the features of the expression of certain genes in the pathological condition compared to the normal condition. Such differentially expressed genes can be involved in the pathogenesis of the disease by changing the quality of their participation in any functional process or signaling pathway. Common approaches to the search for differential gene expression are both the candidate gene approach and large-scale transcriptome analysis, which became possible with the emergence of high-throughput microarray or RNA sequencing technologies-the so-called “omic” studies. Scrutinized literature analysis of gene expression studies (including microarray and mRNA-/tRNA-/lncRNA-sequencing approaches) relevant to VV pathogenesis is presented in two of our works “Differentially expressed genes in varicose vein disease: current state of the problem, analysis of the published data”<sup>[28]</sup> and “Differentially expressed genes in lower limb varicose vein disease”<sup>[29]</sup> that complement each other. For example, a change in the expression level of the following genes was shown repeatedly (according to the results of more than one scientific work) in VVD (the arrow direction corresponds to up- or downregulation): ↑ *VEGF* (*VEGFA*), ↓ *PTGS2* (*COX2*), ↑ *BCL2*, ↑ *BAX*, ↑ *BNIP3*, ↑ *HIF1A*, ↓ *CD31* (*PECAM-1*), ↑ *TGF-β* (*TGFB1*), ↓ *TAGLN* (*SM22-alpha*), ↑ *miR-202* (*microRNA 202*), ↓ *CXCL8* (*IL8*), ↑ *SEPP1* (*SELENOP*), ↑ *RGS4*, ↓ *FOS* (*p55*, *AP-1*, *C-FOS*), ↓ *SOD2* (*Mn-SOD*), ↓ *VCL*, ↑ *ACTC1*, ↑ *TMEM158* (*RIS1*), ↑ *COL15A1*, ↑ *CHRDL2*, ↑ *EFEMP1* and ↑ *TIMP1*. Therefore, these data can be considered reliable. It can be observed that among them are growth factors and mitochondrial proteins, including those involved in apoptosis. Proteins that belong to the extracellular matrix group play an essential role in varicose transformation of the venous wall. It is worth noting that our works also have made a significant contribution to all of this research.

Since the phenotype of a cell or organism as a whole depends on variable gene expressivity, inheritance of the transcriptional status of genes can lead to epigenetic effects. An essential role in the regulation of gene expression is played by epigenetic mechanisms, which include interactions between genetic variants and environmental factors, cellular reactions and pathological processes. DNA methylation is an epigenetic modification that occurs by attaching a methyl (CH<sub>3</sub>) group to the cytosine bases of DNA without changing its sequence, thereby often changing the expression of genes and affecting their function. If we imagine this figuratively in the form of two clothed hands, then, in the case when there is a methyl group, it is a mitten, and, in the absence of it, it is a glove that allows fingers to freely interact (having shown its activity) with transcription factors and the entire transcriptional machine for further regulation of gene expression. In the case of cytosine methylation within the CpG loci, especially if they are concentrated in the regulatory regions of the genome, such as promoters (proximal or distal) or enhancers, “gene shutdown” occurs, i.e., suppression of transcription. In the opposite case, if such a CpG locus is not methylated, then the gene is “turned on”, i.e., it is transcribed; therefore, mRNA from this gene is produced and the protein is also translated (synthesized) in the cell. Hyper- or hypomethylation can cause both inactivation and autoactivation of genes. If the process affects suppressor genes, then the result is the activation of normally suppressed processes. This can serve as a powerful tool for understanding the molecular mechanisms

underlying the disease. Particularly in VVD, the following genes are subjected to hypermethylation: *ADCY3*, *DPEP2*, *HRC*, *PLXNB1* and *MFAP5*<sup>[30,31]</sup>. Hypomethylation of the *CCN5* (*WISP2*) gene in VVs<sup>[30,32]</sup> induces its activity, which, in turn, potentiates TGF- $\beta$ . The result is the remodeling of the extracellular matrix. Moreover, changes in DNA methylation at specific loci as well as other epigenetic marks (histone modifications and non-coding RNAs) are heritable<sup>[33,34]</sup>. For instance, it was demonstrated that genetically identical mice may differ in the degree of their tails' kinkiness, so that mysterious epigenetic marks responsible for variable expressivity are inherited between generations<sup>[35]</sup>.

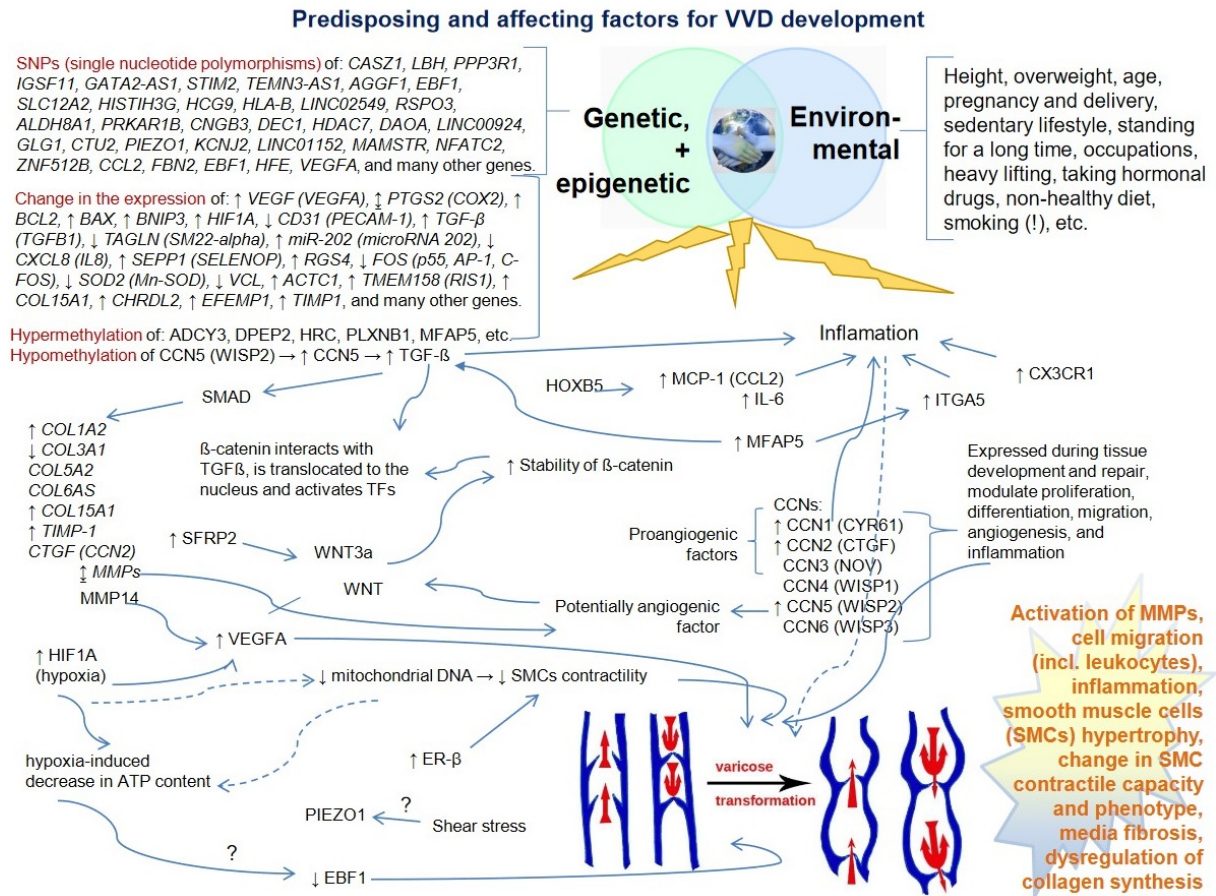
Our study using large-scale microarray analysis of transcriptome and methylome identified sets of differentially expressed genes and differentially methylated DNA loci in VVs compared to non-varicose veins<sup>[30]</sup>. Using independent methods and replicative sample sets, we already validated some of these data. By dint of bioinformatics analysis performed by our colleagues from Germany, the relationship of such master regulators was shown, i.e., those genes whose products are likely to participate in the pathogenesis and possibly trigger the processes of vein wall remodeling and its varicose transformation. These are genes for the extracellular matrix organization (including collagens and tissue inhibitors of metalloproteinases), cell adhesion and vascular morphogenesis. [Figure 1](#) schematically represents (of course, not completely) the interplay of the genetic master regulators contributing to VVD development.

#### **WHERE ARE WE NOW IN TERMS OF LEARNING, IN TERMS OF UNDERSTANDING, AND WHERE ARE WE GOING? A CHALLENGE FOR THE FUTURE**

Molecular events occurring in the pathogenesis of VVs are indeed known fragmentarily, and their sequence is not always clear: what is the cause and what is the consequence. Many of the heroes involved in certain events are still behind the scenes. By studying the features of gene expression and methylation in the pathological condition, as well as other processes, researchers are able to decipher the mechanisms and various stages of pathogenesis step by step.

Realizing that, after all, the pathogenesis of chronic venous diseases is a very complex process, it should be borne in mind that even a single gene effect can cause little influence. Therefore, studies with a large number of patients are required before drawing any conclusions. In addition, the impact of a particular polymorphism will depend on genotype-environment interactions that may be specific to a given patient population. Genome-wide research has indeed made a significant contribution to the discovery of genes associated with venous pathology, but there are still puzzles that need to be pieced together. Multicenter international studies and the formation of consortia will help to clarify the connection between genetic markers and various pathological components of chronic venous diseases, including VVD. When efforts come together, these will not only be large samples, but they will also be synchronized according to inclusion-exclusion criteria, research methodology and interpretation of the analysis results, and they will take into account the ethnicity of the analyzed groups.

It is expedient to draw the attention of readers to the following fact: the absence of the relative levels of genes' expression in the veins in relation to other organs and tissues. In one of the largest databases of biological and medical research, NCBI (PubMed/Gene), there is no information on the levels of gene expression, not only in the veins but even in the vessels! Only 27 organs and tissues are indicated there (heart, lungs, liver, kidneys, etc.). In another database-UCSC Genome Browser on human-which provides the gene expression profiles in 54 organs and tissues, one can already find information on gene expression in the arteries (however, only in three kinds-aorta, coronary and tibial arteries), but the veins are not there yet. If we imagine it is possible to add new missing data, then in perhaps the future it will be possible to get a more complete picture. Of course, such voluminous studies on large samples of people require large



investments and efforts of not one person, but collectives. However, without such data for veins (relative to other organs and tissues), further research on venous-specific action, for example, of certain medications, will be very difficult. Recently, it was shown that mitochondrial DNA copy number (mtDNA-CN) was inversely associated with incident and prevalent cardiovascular disease outcomes in 21,870 participants from three independent cohorts<sup>[36]</sup>. Shortly after, in our laboratory, we found that mtDNA-CN was decreased in varicose vein vs. non-varicose vein tissue samples<sup>[37]</sup>. Then, Castellani *et al.*<sup>[38]</sup> demonstrated across multiple independent cohorts that changes in mtDNA-CN influence nuclear DNA methylation at specific CpG loci and result in differential expression of specific genes that may impact human health and disease (namely, cardiovascular disease and all-cause mortality) via altered cell signaling<sup>[38]</sup>. Understanding the mechanisms of mitochondrial and nuclear communication may shed light on the complex etiology of the disease, as well as the implications of therapeutic strategies to increase mitochondrial function. The fact that some drugs used in VVD treatment show their benefits in treating arterial diseases as well may serve as auxiliary evidence for the common molecular-genetic pathways in the pathogenesis of vascular diseases. A significant phlebotonic effect seems to be improbable without any impact on arterial smooth muscles and blood pressure<sup>[39]</sup>. Indeed, horse chestnut extract contracts (through serotonin receptors, at least partly) both veins and arteries, but it is more potent in inducing venocontraction<sup>[40]</sup>. A pharmacological vasoactive agent, diosmin, is capable of inhibiting inflammatory pathways since it simultaneously influences pro-angiogenic/anti-angiogenic balance by an increase of anti-angiogenic factors and reducing the level of pro-angiogenic factors in blood plasma (TNF-alpha, VEGF-A and VEGF-C, angiostatin, IL-6, FGF2 and

PLG)<sup>[41]</sup>. Bioflavonoids found in citrus bioflavonoid extract, grape seed extract, pine bark extract and green tea extract participate in the regulation of human aortic smooth muscle cell-mediated contraction and have a strong potential for counteracting pathophysiological effects of angiotensin II-the leading mediator of clinical systemic hypertension<sup>[42]</sup>. Anti-hypertensive effects of diosmin (namely, compromised NO-synthase inhibitor induced cardiac infarcts, hyaline arteriopathy and fibrinoid necrosis), presumably because of its activity in elimination of superoxide anions, were demonstrated in a rat model<sup>[43]</sup>. Peripheral arterial disease may also be treated with Sulodexide, which prevents cardiovascular events after myocardial infarction and exerts the relief of intermittent claudication<sup>[44]</sup>.

In 2020, a Russian group of scientists performed a scrutinizing review based on the literature data and their own works and postulated that: (a) primary varicose transformation of superficial veins develops as a result of remodeling of their walls; (b) vein wall remodeling is based on a complex of molecular processes determined by genetic predisposition; (c) vein wall remodeling is reversible, i.e., the dilated and altered vein can return to its original (“healthy”) state; and (d) varicose veins can probably be successfully cured pharmacologically with no surgical interventions needed<sup>[45]</sup>. **Table 1**, compiled on the basis of data from Kharkevich (2004)<sup>[39]</sup>, Maggioli<sup>[46]</sup> (2016), Ramelet *et al.*<sup>[47]</sup> (2017) and Mansilha and Sousa<sup>[48]</sup> (2018), as well as other sources, is a kind of generalization of the treatment options for patients suffering from chronic venous diseases.

Apparently, not all the works devoted to the investigation of phlebotonics’ efficacy are cited in this table. Martinez-Zapata *et al.*<sup>[72]</sup> performed a very thorough (237 pages) review “Phlebotonics for venous insufficiency” on randomized, double-blind, placebo-controlled trials where the efficacy of phlebotonics (rutosides, diosmine, hidrosmine, calcium dobesilate, aminaftone, disodium flavodate, chromocarbe, *Centella asiatica*, French maritime pine bark extract and grape seed extract) was assessed. The authors concluded that there was “moderate-certainty evidence that phlebotonics probably reduce (o)edema slightly, compared to placebo; moderate-certainty evidence of little or no difference in quality of life; and low-certainty evidence that these drugs do not influence ulcer healing; moderate-certainty evidence suggests that phlebotonics are probably associated with a higher risk of adverse events than placebo”<sup>[72]</sup>. One possible reason that the desired efficacies were not observed in many clinical trials could be because, in order to have an effect, many flavonoids should be metabolized due to the intestine microflora<sup>[46,43]</sup>. What if the patient’s microflora is impaired? Does that mean all those efforts might be in vain? It is known that one of the most popular drugs in phlebological practice - micronized purified flavonoid fraction - is more effective as a complex substance rather than its components applied separately<sup>[73]</sup>. Nowadays, new possible venoactive drugs are being investigated. Recently, using a mouse model, Lust *et al.*<sup>[74]</sup> showed that diclofenac (COX-2, or cyclooxygenase, inhibitor) is capable of attenuating venous remodeling *in vivo*<sup>[74]</sup>. We are likely entering the era of research on new potential substances targeting the main drivers of pathological processes in varicose vein disease.

## CONCLUSION

In recent decades, the studies on varicose veins have paid much attention to its genetic constituent. In this perspective, we provide a critical overview and literature analysis on the heredity of varicose veins, genetic association studies, gene expression and epigenetic studies and point out the gaps existing in the field. We hope that the generalized information given will be useful not only for fundamental scientific researchers, but also for clinicians, and will be further used in phlebological practice. Revealing the genetic basis of venous diseases can help to clarify the mechanisms of pathogenesis, and their understanding, in turn, can help to develop new methods of prevention and treatment of varicose veins. Of note, for disease risk assessment, epigenetic factors and their heritability should be considered as well. Perhaps in our century



**Table 1. Main venoactive drugs (venoprotective and venoconstrictor/venotonic) used for CVD<sup>1</sup> treatment**

Substance [Grade <sup>2</sup> , if available]	Group	Registered as: trade name [country]	Effects	Ref.
I. Mixed-action drugs (venoprotective and venoconstrictor/venotonic)				
Micronized purified flavonoid fraction (MPFF) [A]	90% micronized diosmin and 10% flavonoids (hesperidin, diosmetin, linarin, and isorhoifolin)	Daflon <sup>®</sup> [France], Detralex <sup>®</sup> [France, Russia], Detravenol [Russia], Venarus [Russia], Angiorus [Russia], Linfadren [Italy]; Ardium [France], Alvenor [Bangladesh], Arvenum [Italy], Capiven [Denmark], Elatec <sup>®</sup> [Mexico], Variton <sup>®</sup> [Mexico], Flebotropin [Argentina], Venitol <sup>®</sup> [South Korea], VEDIPAL <sup>®</sup> [Argentina, Chile, Puerto Rico, Spain, Guatemala, etc.], Venolex [Georgia], Flebaven <sup>®</sup> [Russia]	Increases venous tone, protects against inflammation-related valve damage, decreases capillary permeability, improves capillary resistance, increases lymphatic drainage and alleviates leg (o)edema, improves skin trophic disorders and ulcer healing, relieves symptoms related to CVD <sup>1</sup> at all stages and improves QoL; more effective as a complex substance rather than its components applied separately	[43,46,49-55]
Diosmiplex	Diosmin glycoside (flavonoid) + Alka4-complex	Vasculera <sup>™</sup> (USA)	Restores toward normal the metabolic aspects of CVI including modulation of venous tone and capillary resistance, management of lymphatic drainage, and inflammation in the microcirculation; reduces acidosis under extreme exercise loads	[56-59]
Diosmin [C]	Flavonoid	Phlebodia <sup>®</sup> 600* [France], Diovenor <sup>®</sup> [France], Dio-PP [Hungary], VENO V [Portugal], Venosmine [Italy, Hong Kong]	Prolongs the vasoconstrictor effect of norepinephrine on the vein wall, increases venous tone, reduces venous capacitance, distensibility, and stasis; improves lymphatic drainage; improves all CEAP stages of CVD including venous ulcers, and improves QoL	[41,43,60,61]
Horse chestnut seed extracts [B]	Saponosides	Horse chestnut extract fluid [Russia], Escizane and Aescine [Russia], Aescin (escin) [Poland, Slovakia, Taiwan], Esceven (escin, heparin) [Poland], Aescusan 20 [Germany], Aescin-Teva [Czech Republic], Reparil <sup>®</sup> (diethylamine salicylate, escin) [Italy, Belgium, Brazil, etc.], Aescusan [Germany], Aesflazidum [Russia], Venoplant <sup>®</sup> [Germany], Venostasin [Austria], Anavenol Zentiva (dihydroergocristine mesylate + esculin <sup>†</sup> + rutin) [Czech Republic]	Anti-inflammatory, anti-(o)edematous, and venotonic effects; may be an alternative for compression stockings	[40,60,62,63]
Ruscus extracts [B]	Saponosides; Ruscosides (Butcher's broom)	Cyclo-3 fort (broomstick extract + hesperidin methylchalcone + ascorbic acid) [Poland], Phlebodril [Germany]	Antioxidant and anti-inflammatory properties, as well as significant venular constriction effect	[64,65]
Grape Seed Extract [C]	Procyanidolic oligomers, precursors of tannins	Endotelon [France], AS195, Antistax <sup>®</sup> (red vine leaf extract) [Germany]	Due to its anti-oxidant properties can strengthen blood vessels and reduce inflammation; maintains slide circulation and improves the tired heavy achy feeling that results from venous insufficiency	[63,66,67]
Glucofuranoside derivative	Synthetic drug	Glyvenol <sup>®</sup> (tribenoside) [Switzerland, Germany]	Anti-inflammatory and analgesic effects; decreases capillary permeability	[47]
II. Venoprotective drugs				
Rutin and its derivatives (oxerutin [A]; troxerutin [C])	Bioflavonoids; rutosides	Rutin (rutoside), Ascorutin [Russia, Slovakia, Georgia, Czech Republic], Troxevasin <sup>®</sup> (troxerutin) [Bulgaria], Cerutin [Lebanon, Poland], Rutoscorbin [Lithuania, Poland, Romania]	Reduces leg (o)edema; improves CVD symptoms	[47,48,60,63]
Ginkgo biloba leaf extracts [C]	Quercetol, rutoside (leaf)	Bilobil, Ginkjo, Memoplant; Ginkor Fort (Ginkgo biloba extract + heptaminol <sup>‡</sup> hydrochloride + troxerutin) [France]	Reduces leg (o)edema; improves CVD symptoms	[46-48]

Calcium dobesilate [A]	Dioxybenzene derivative (synthetic drug)	Doxium (calcium dobesylate) [Serbia, Russia], Evadol and Proxacin [Peru], Danium [Czech Republic], Calcium dobesilate Galena [Poland]	Reduces leg (o)edema; improves CVD symptoms.	[47,48]
III. Other venoactive drugs				
Sulodexide	Porcine-derived extracts of intestinal mucosa (two glycosaminoglycans: 80% of fast-moving heparin and 20% dermatan sulfate)	Vessel [Italy], Aterina [Spain], Vessel <sup>®</sup> Due F [Russia and all over the world]	Reduces peripheral venous pressure, improves both objective and subjective CVD symptoms and QoL, improves ulcer healing, prevents recurrent venous thromboembolism	[44,68-70]
Gotu Kola (Centella asiatica)	Saponosides	Madecassol [Greece, Portugal, Turkey], Centellase [Italy]	Stimulates collagen production, reduces swelling and improves circulation; is used to treat venous insufficiency and heal wounds	[71]

<sup>§</sup>Grade of Recommendations (according to Ramelet *et al.*<sup>[47]</sup>) depends on the conduction of the Randomized Controlled Trial (RCT): (a) RCT with large sample sizes, valid meta-analyses (Grade A); (b) RCT with small sample size (Grade B); and (c) other controlled trials, no RCTs (Grade C). <sup>†</sup>Esculin is a coumarin glycoside from the leaves and bark of horse chestnut (glycone - glucose, aglycone - 6,7-dioxycoumarin). <sup>‡</sup>Heptaminol hydrochloride tones up varicose veins and has a cardio-stimulating effect. Some venoconstrictor/venotonic only drugs [such as  $\alpha$ -adrenergic agonists fentanyl (Ethylephrine; Actiq, Duragesic, Sublimaze) and midodrine [Gutron; Orvaten, ProAmatine], dihydrogenated ergot alkaloids (Dihydroergotoxin, Dihydroergotamine, Dihydroergocriptine, Vazobral)] were not included in this table due to their irrelevance for CVD treatment. <sup>¶</sup>CVD: Chronic venous disease. QoL: quality of life. CVI: chronic venous insufficiency.

some miracle remedy will be invented, even if not universal, but which will really be able to help patients.

## DECLARATIONS

### Acknowledgments

The authors are grateful to the native English speakers Holly Jacob (Texas A&M University, USA) and Christine Tierney (Houston Community College, USA) for grammar checking assistance and thorough text revision.

### Authors' contributions

Made substantial contributions to conception and design of the study: Smetanina MA, Shevela AI, Gavrilov KA, Filipenko ML

Performed literature analysis and writing the manuscript: Smetanina MA

Helped with data interpretation relating to clinical part: Shevela AI, Gavrilov KA

Helped with data acquisition, as well as provided administrative, technical, and material support: Filipenko ML

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

This work was supported by the Program of Fundamental Scientific Research of the Russian Federation (PFSR RF) “Fundamental Basics of Health Preservation” (No. 121031300045-2).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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