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Research Article

Identifying potential novel biomarkers for varicocele: A bioinformatics approach to genomics analysis

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Abstract

Introduction: Varicocele, characterized by the enlargement of scrotal veins, is a common contributor to male infertility, but its genetic underpinnings remain largely unknown.

Aim: The goal of this study is to identify potential biomarkers associated with varicocele in order to better understand its molecular mechanisms.

Materials and methods: Using the three primary databases, NCBI, DisGeNET, and OpenTarget, we analyzed gene variants and found 79 pertinent genes associated with varicocele. Protein-protein interaction analysis was performed using STRING and visualized with Cytoscape. Molecular Complex Detection (MCODE) and CytoHubba tools helped identify significant protein clusters.

Results: The gene ontology analysis shows that there are 79 proteins involved in the inflammatory process, the regulation of gene expression, and cellular components that play a role in oxidative stress and angiogenesis. Our results revealed three key biomarkers: Interleukin-1 beta (IL1B), B-cell lymphoma 2 (BCL2), and matrix metalloproteinase-9 (MMP-9). These proteins are involved in critical processes, such as inflammation, oxidative stress, angiogenesis, and vascular damage, that are central to the pathophysiology of varicocele.

Conclusion: The identification of IL1B, BCL2, and MMP-9 offers new insights into varicocele's molecular mechanisms and suggests potential targets for diagnostic and therapeutic strategies, advancing personalized treatment approaches for fertility restoration.

Keywords

BCL2, bioinformatics, IL1B, MMP-9, varicocele

Introduction

Varicocele is a medical condition in which men's scrotum veins widen, potentially interfering with testicular hemodynamics.[1] It is estimated to affects 15% to 20% of the world's male population, and is thought to be responsible for more than 40% of male infertility.[2,3] Varicocele has a significant impact on the quality of sperm and semen. This condition is caused by instability of the hemodynamics.[4,5] DNA fragmentation is also known to play a big role in the development of varicocele, which is caused by the process of apoptosis, packaging abnormal chromatin, as well as oxidative stress.[6] In addition, varicocele is known to damage sperm DNA function through improved oxidative stress and hypoxic effects on the testes.[7]

Various risk factors have been identified in the development of varicocele, such as damage to the venous valves, physical activity, weight, hormonal imbalance, smoking

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habits, and genetic factors.[8,9] Patients with varicocele are often underdiagnosed, either when presenting with pain or during fertility evaluations.[10] Although most cases of varicocele are asymptomatic, some patients present with a feeling of heaviness in the scrotum or even testicular atrophy.[1]

Mutation genetics, such as single nucleotide polymorphism (SNP), are also linked with varicocele and infertility.[11] Previous research has shown that varicocele is the only component of strong genetics, with evidence that the condition can be naturally derived, indicating that genetics play an important role in the development of this disease.[12,13] One of the interesting findings is the improvement in the expression of the enzyme nitric oxide synthase (NOS) in patients' varicocele, which causes relaxation in cells, muscles, and blood vessels.[14] Furthermore, the development of varicoceles is influenced by endothelial vascular overload, which is mediated by prokineticin 2 (*PK2*) and a number of other genes that contribute to varicocele pathophysiology.[15,16]

Despite these findings, the genetic basis of varicocele remains poorly understood. Current studies have mainly focused on clinical manifestations and general pathophysiological mechanisms, while the role of specific genetic biomarkers, particularly genes involved in oxidative stress and angiogenesis, is still underexplored and not clearly validated.[17] This lack of precise molecular understanding contributes to delayed diagnosis and limited options for targeted therapy. Therefore, further investigation into genetics associated with varicocele is essential to establish reliable biomarkers that may improve early detection, risk stratification, and personalized treatment strategies. Various genome database platforms are used to investigate single nucleotide polymorphisms (SNPs) that have the potential to regulate angiogenesis and oxidative stress, making them biomarkers and treatment targets.[18-20]

Aim

While the results from this study are still limited, the findings provide valuable insights into the potential role of genetic biomarkers in the diagnosis and treatment of varicocele. The aim of this study was to look into genes that may influence the development of varicoceles in the hopes of laying the groundwork for future research. This could eventually lead to the discovery of new biomarkers for diagnosing varicocele that can be used as therapeutic targets in the future.

Materials and methods

Identification of variant genes associated with varicocele

This study used three different database platforms, all of which were accessed on February 22, 2025: OpenTarget (https://platform.opentargets.org/), DisGeNET (https://www.disgenet.com/), and NCBI. The gene variants associated with varicocele were identified by searching the term "varicocele" on each platform to gather relevant genes. The screening process was based on specific criteria: genes with an OpenTarget score greater than 0.03, a DisGeNET score greater than 0.3, and genes expressed in Homo sapiens for NCBI.[21]

Discovery of varicocele gene biomarkers

Following the identification of key genes, protein-protein interaction (PPI) analysis was conducted to further explore the biological relationships among the encoded proteins. This analysis utilized the STRING database (https://string-db.org/), accessed on February 22, 2025, which provides comprehensive information on known and predicted functional protein interactions. The resulting PPI networks were visualized using Cytoscape software, enabling a clear representation of the biological interactions. To identify significant protein clusters, the Molecular Complex Detection (MCODE) plugin in Cytoscape was applied with the following parameters: degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max depth = 100. Subsequently, to determine the most central and potentially influential proteins within the network, the CytoHubba plugin was employed using the Maximal Clique Centrality (MCC) algorithm, which is recognized for its accuracy in identifying hub proteins. The top ten ranked nodes were selected, and the three highest-scoring proteins were proposed as potential biomarkers associated with varicocele due to their central role in the interaction network.

Biological pathway analysis and functional roles

To investigate the biological mechanisms involved, the STRING database was accessed on February 22, 2025, to analyze protein-protein interactions. Biological pathway analysis was conducted using WebGestalt, which integrates the KEGG database to link genomic data with high-level functional insights, with significant enrichment determined at a q-value (FDR) cutoff of 0.05. The enrichment results were visualized using a bubble node graph to facilitate interpretation of the connections between various biological components. Additionally, gene function was assessed through Gene Ontology (GO) analysis, which classifies genes into three main categories: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). The outcomes of the GO analysis were presented in bar charts to illustrate the functional distribution of the identified target genes in this study.

Results

Identification of variant genes associated with varicocele

Gene screening was carried out using specific inclusion criteria: genes with a relevance score greater than 0.03 in OpenTarget, greater than 0.3 in DisGeNET, and genes restricted to Homo sapiens, according to NCBI. The identification of gene variants associated with varicocele was conducted through three major databases: OpenTarget, DisGeNET, and NCBI, accessed on February 22, 2025. A search using the keyword "varicocele" yielded 415 genes from OpenTarget, 204 from DisGeNET, and 93 from NCBI. After applying the filtering criteria, 25 genes from OpenTarget, 4 from DisGeNET, and 58 from NCBI met the specified thresholds and were specific to Homo sapiens. Removing duplicates, a total of 79 unique genes were identified **(Table 1)**. These findings provide an initial set of candidate genes potentially associated with varicocele, serving as a basis for further functional and network analyses.

Table 1. Varicocele-associated genes

*DNMT*1	*ARE*	*MMP-9*	*CST3*	*CACNA1H*
NLRP3	*NOS2*	*PTGS2*	*CHI3L1*	*PEPD*
BRD2	*SLC7A11*	*GSTM1*	*FASLG*	*GNLY*
TRPV1	*INSL3*	*BCL2*	*SHH*	*HSPA2*
TLR4	*IL-1A*	*MTOR*	*AMH*	*ARG2*
PROK2	*CATSPER1*	*GSTT1*	*MIR210*	*SERPINA5*
PPARA	*DNMT3B*	*IGF1*	*NOS1*	*SRD5A1*
IL1B	*VEGFA*	*GSTP1*	*RARA*	*PLCZ1*
PLOD1	*FASN*	*HMOX1*	*NT5E*	*CACNA1G*
EGF	*CASP9*	*PARP1*	*BAK1*	*FGL2*
FN1	*TP53*	*ESR2*	*PTGS1*	*NAIP*
PHGDH	*TGFB1*	*FAS*	*EDNRB*	*PRM1*
DNMT3A	*MTHFR*	*EDN1*	*AGTR2*	*PRM2*
LEP	*NOS3*	*TNFSF10*	*EDNRA*	*MIR210HG*
HIF1A	*ESR1*	*BAX*	*TXNRD1*	*ROPN1*
CASP1	*ACE*	*AHR*	*INHBB*	

Discovery of varicocele gene biomarkers

Following the identification of genes associated with varicocele, their corresponding proteins were analyzed using the STRING database to investigate functional associations. The resulting Protein-Protein Interaction (PPI) network consisted of 76 nodes and 594 edges, with an average node degree of 15.6 and an average local clustering coefficient of 0.68. The network showed statistically significant enrichment (p<1.0e-16), suggesting that the observed interactions are unlikely to occur by chance and are functionally meaningful. To identify highly interconnected sub-networks, the MCODE plugin in Cytoscape was utilized, resulting in five distinct protein clusters, each exhibiting unique topological characteristics in **Fig. 1**. These clusters coded visually as red diamonds (cluster 1), orange ellipses (cluster 2), green polygons (cluster 3), gray octagons (cluster 4), and purple triangles (cluster 5) are potential biomarker modules in varicocele-related molecular networks.

Further prioritization of key regulatory proteins was carried out using the CytoHubba plugin with the Maximal Clique Centrality (MCC) algorithm, which identified the top ten hub proteins **(Fig. 2)**. These were visualized using color gradients from dark red to light red, representing decreasing centrality values. Among these, IL1B, BCL2, and MMP-9 emerged as the most central proteins and are proposed as potential biomarkers for varicocele diagnosis or targeted therapeutic development.

Biological pathway analysis and functional roles

To further explore the biological significance of these proteins, functional and pathway enrichment analyses were conducted. STRING-based KEGG pathway analysis revealed 115 enriched biological pathways, with the top three being pathways in cancer, platinum drug resistance, and the HIF-1 signaling pathway. These findings suggest a link between varicocele and oxidative stress responses, as well as cellular mechanisms commonly implicated in tumorigenesis. Gene Ontology analysis provided additional insights into the functional roles of the proteins. A total of 1,205 biological processes (BP) were enriched, with the most significant being blood pressure regulation, response to hypoxia, and response to oxygen levels highlighting the relevance of these pathways in varicocele-related vascular and metabolic dysregulation. In the Molecular Function (MF) category, 55 functions were identified, notably including transcription coactivator binding, nuclear receptor activity, and DNA methyltransferase activity, which indicate involvement in gene expression control and epigenetic regulation. Meanwhile, the Cellular Component (CC) analysis identified 29 significant components, such as the inflammasome complex, voltage-gated calcium channel complex, and platelet alpha granules, pointing to the role of inflammation and vascular pressure modulation in varicocele pathophysiology **(Fig. 3)**.

Discussion

Varicocele is still a medical condition with unknown causes, though genetic factors are becoming more widely recognized as influencing its development.[20] This study aimed to identify key genetic biomarkers associated with varicocele using integrated bioinformatics approaches, including STRING, MCODE, and CytoHubba, which have been effectively applied in related conditions such as male infertil-

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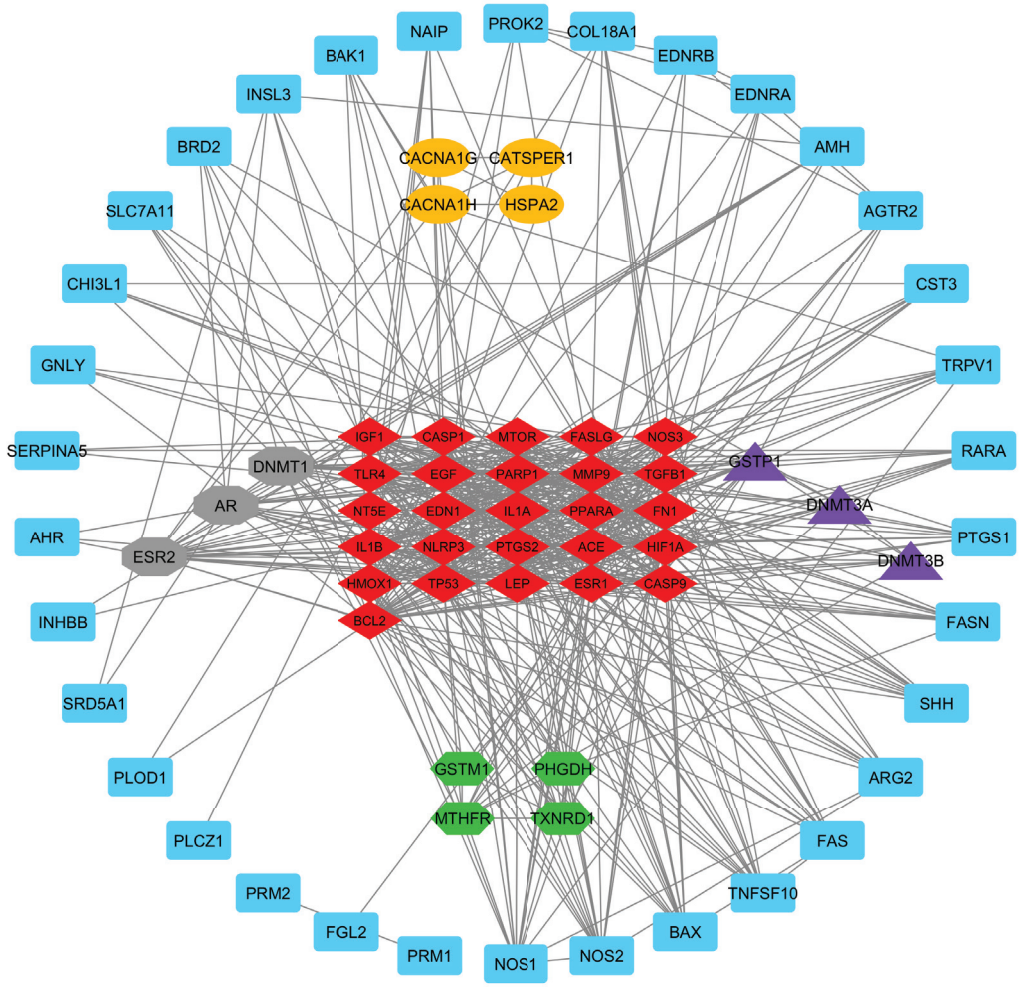

Figure 1. Construction of a Protein-Protein interaction (PPI) network for genes associated with varicocele.

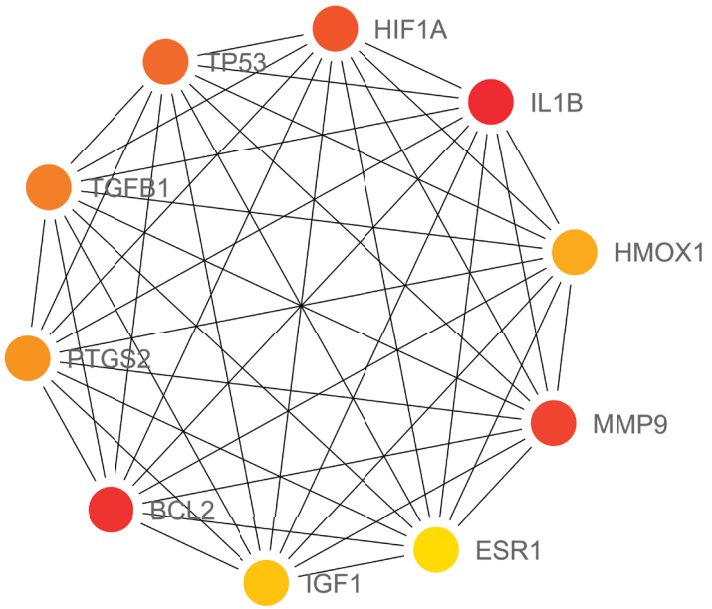

Figure 2. Higher degree values are represented by red to yellow colors. The rank of genes was identified from CytoHubba. The higher the score, the higher the rank of the biomarker.

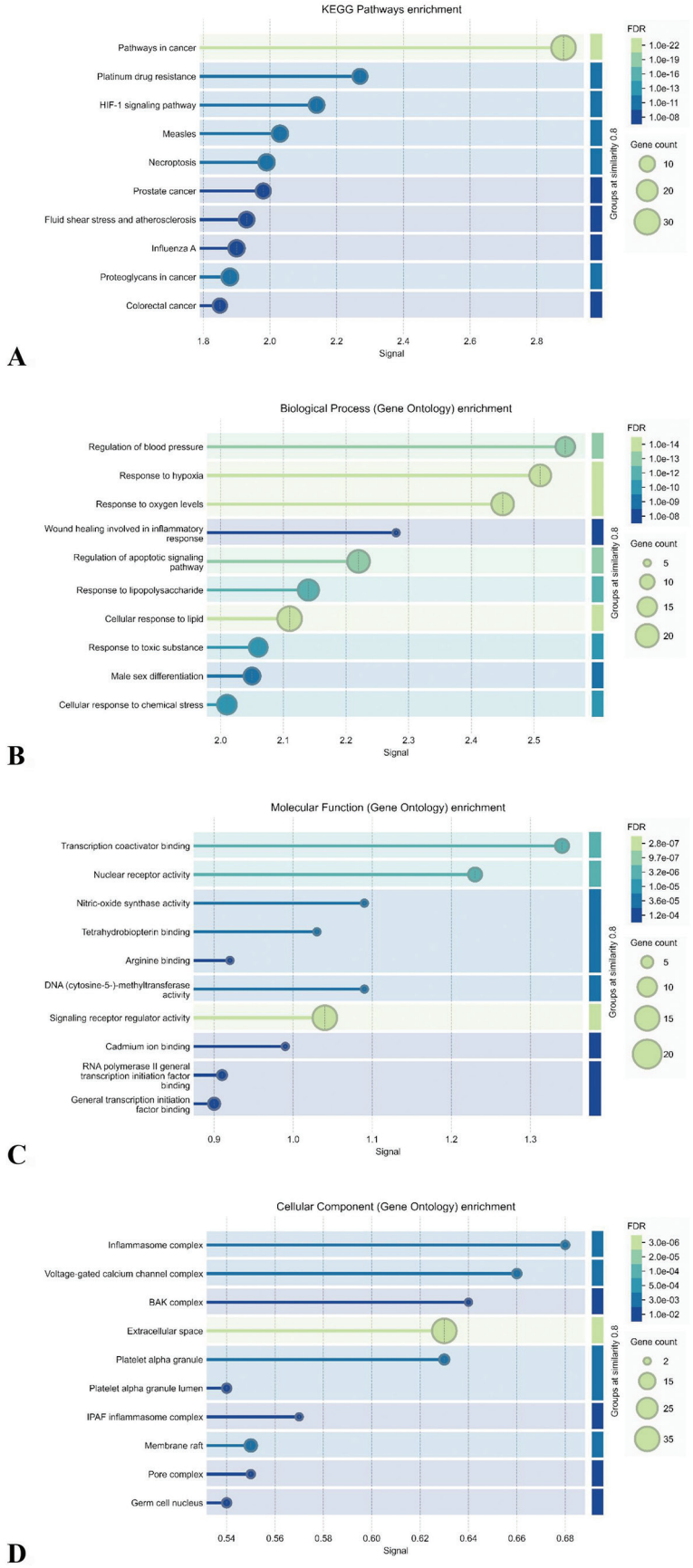

Figure 3. Visualization of the top 10 biological pathways and functional roles: (**A**) KEGG pathway, (**B**) biological processes, (**C**) molecular function, (**D**) cellular components.

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ity and liver fibrosis.[21,22] Our analysis identified three significant candidate biomarkers: IL-1B, BCL2, and MMP-9.

Interleukin-1 beta (IL-1B) is a proinflammatory cytokine critically involved in vascular pathology, including varicocele, characterized by abnormal venous dilation in the scrotum. It mediates inflammatory signaling through the NF-κB pathway, contributing to endothelial dysfunction and vascular inflammation observed in related diseases like atherosclerosis.[23] Such mechanisms plausibly underpin varicocele progression via chronic vascular inflammation and increased endothelial permeability, which may exacerbate venous damage.[24] Additionally, IL-1B promotes oxidative stress by stimulating reactive oxygen species (ROS) production, impairing endothelial function and exacerbating venous hypertension typical of varicocele.[25] Its role in angiogenesis, through upregulation of VEGF in macrophages, further implicates IL-1β in abnormal vessel formation.[26] These multifaceted functions position IL-1B as a promising biomarker and potential therapeutic target, especially for inflammation-driven varicocele.

B-cell lymphoma 2 (BCL-2), an anti-apoptotic protein, regulates cell survival and is linked to angiogenesis, oxidative stress, and autophagy. Increased BCL2 expression has been observed in spermatic veins of varicocele patients, suggesting enhanced endothelial cell survival that may contribute to pathological angiogenesis and venous abnormalities.[27] The imbalance between BCL2 and pro-apoptotic BAX exacerbates oxidative damage to vascular and testicular tissues, potentially impairing sperm quality and fertility.[28] Furthermore, BCL2's interaction with hypoxia-inducible factors such as HIF-1α[29] reflects a cellular adaptive response to hypoxic stress caused by disrupted blood flow, stimulating angiogenesis and cell proliferation.[30] These insights highlight BCL2's critical role in maintaining venous homeostasis under varicocele-associated stress.

MMP-9 is a matrix metalloproteinase involved in extracellular matrix remodeling critical for vascular integrity.[31] Genetic polymorphisms in MMP-9 have been linked to varicose veins, a pathophysiologically related condition, and may similarly predispose individuals to varicocele through altered extracellular matrix degradation leading to vessel dilation and valve incompetence.[32] MMP-9 activation by ROS generated under heat stress and venous congestion damages collagen and elastin fibers, reducing vascular elasticity and exacerbating varicocele progression.[32] Moreover, dysregulated MMP-9 activity disrupts angiogenesis, leading to inefficient collateral vessel formation and persistent venous congestion. The imbalance between MMP-9 and its inhibitor TIMP2 further aggravates this pathological process, underscoring MMP-9's central role in varicocele pathogenesis.

Previous studies have investigated the genetic underpinnings of male infertility, either through targeted analysis of specific polymorphisms, such as the NOS3 variants linked to oxidative stress in varicocele patients, or by identifying broader infertility-associated genes using genome-wide bioinformatics approaches.[33] However, these efforts did not specifically isolate or prioritize genetic biomarkers uniquely relevant to varicocele as a distinct pathological entity. The bioinformatics study, for instance, focused on general male infertility without distinguishing varicocele-associated molecular mechanisms, identifying genes like TEX11 and SYCP3 primarily involved in meiosis and spermatogenesis. While previous studies have noted the involvement of these proteins in vascular disorders, our integrative bioinformatics approach uniquely prioritizes IL1B, BCL2, and MMP-9 as varicocele-specific biomarkers by combining multi-database gene screening with network and functional analyses. This provides novel insights into their potential interplay in varicocele development, bridging gaps between genetic predisposition and molecular mechanisms. However, this study's reliance on in silico data limits direct clinical applicability without experimental validation.

This study's limitations include its reliance solely on computational bioinformatics analysis without experimental validation, which limits the ability to confirm the actual expression or functional impact of the identified biomarkers IL1B, BCL2, and MMP-9 in varicoceles. The absence of patient-derived clinical samples and functional assays means that the biological roles of these genes in varicocele pathogenesis remain hypothetical. Additionally, the cross-sectional design limits interpretation of causality and dynamic gene expression changes over time or in response to treatment. To address these limitations, future studies should include molecular validation using patient tissues, assess biomarker specificity through case-control comparisons, and apply longitudinal designs to evaluate changes post-varicocelectomy. Incorporating predictive modeling tools such as ROC analysis and regression frameworks would also help determine the diagnostic and prognostic utility of these candidate genes in clinical practice.

Conclusion

This study successfully identified 79 varicocele-associated genes by integrating data from OpenTarget, DisGeNET, and NCBI databases. Subsequent protein-protein interaction network analysis and module detection pinpointed three key biomarkers, IL1B, BCL2, and MMP-9, which are critically involved in varicocele pathogenesis through mechanisms such as inflammation, oxidative stress, angiogenesis, and vascular damage. These biomarkers hold promise for improving varicocele diagnosis and developing targeted therapeutic strategies. Overall, our findings enhance the molecular understanding of varicocele and lay the groundwork for future research aimed at more precise and effective clinical interventions.

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Author contributions

M.F. conceptualized and designed the study, conducted the majority of data analysis, interpreted the results, and wrote the original draft of the manuscript. H.F.A. assisted in data collection, contributed to the bioinformatics analysis, and participated in reviewing and editing the manuscript. Both authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Competing interests

The authors have declared that no competing interests exist.

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