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Original article

Integrated genomic network analysis revealed potential of a druggable target for hemorrhoid treatment

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ARTICLE INFO

Keywords:
Drug repositioning
Genomic network analysis
Hemorrhoids
Sapropterin

ABSTRACT

Hemorrhoids are a prevalent medical condition that necessitates effective treatment options. The current options for treatment consist of oral medications, topical applications, or surgery, yet a scarcity of highly effective drugs still exists. Genetic markers provide promising avenues for investigating the treatment of hemorrhoids, as they may reveal intricate biological mechanisms and targeted drug therapies, ultimately enhancing more precise treatment tailored to the patient. This study aims to identify new drug candidates for treating hemorrhoids through a meticulous bioinformatics approach and integrated with genomic network analysis. After extracting 21 druggable target genes using DrugBank from 293 genes connected to hemorrhoids, 87 possible drugs were selected. Three of these drugs (ketamine, methylene blue, and fulvestrant) hold potential in addressing issues associated with hemorrhoids and have been supported by clinical or preclinical studies. Eighty-four compounds present new therapeutic possibilities for managing hemorrhoids. We highlight that our findings indicate that *NOX1* and *NOS3* genes are promising biomarkers, with *NOS3* gaining significance owing to its robust systemic functional annotations. Sapropterin, an existing drug, is closely associated with *NOS3*, providing a clear target for biomarker-driven interventions. This study illustrates the potential of combining genomic network analysis with bioinformatics to repurpose drugs for treating hemorrhoids. Subsequent research will explore the mechanisms for utilizing *NOS3* targeting in the treatment of hemorrhoids.

1. Introduction

Hemorrhoids, a prevalent medical condition, are characterized by inflammation and swelling of blood vessels encircling the rectal and anal regions. This ailment arises from heightened vascular pressure induced by factors such as pregnancy, aging, and persistent constipation (Sardiñas et al., 2016; Sun and Migaly, 2016). While not life-threatening, hemorrhoids result in discomforting symptoms, including pain, itching, bleeding, and thrombosis (Sanchez and Chinn 2011). Pathological changes linked to hemorrhoids encompass abnormal vein dilation, vascular thrombosis, collagen degradation, fibroelastic tissue transformation, and structural disruption of subepithelial anal muscles (Lohsiriwat 2012). An exhaustive analysis of surgical specimens from hemorrhoidectomy patients revealed a significant inflammatory response impacting vascular walls and connective tissue (Lohsiriwat 2015).

Determining the prevalence of hemorrhoids remains intricate due to numerous asymptomatic patients avoiding medical consultation. Remarkably, research involving colorectal cancer screening identified hemorrhoid prevalence in 39 % of participants, with 55 % being

Peer review under responsibility of King Saud University.
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https://doi.org/10.1016/j.jsps.2023.101831
Received 19 August 2023; Accepted 14 October 2023
Available online 20 October 2023

asymptomatic (Riss et al., 2012). This underscores the need for innovative therapeutic avenues. Hemorrhoids predominantly affect individuals aged 45 to 65 and correlate with conditions that elevate pressure on the hemorrhoidal venous plexus, such as straining during defecation due to constipation (Chong and Bartolo 2008; Riss et al., 2012). Additional factors, including obesity, pregnancy, chronic diarrhea, anal intercourse, cirrhosis with ascites, pelvic floor dysfunction, and a low-fiber diet, contribute to hemorrhoid development (Chong and Bartolo 2008; Jacobs 2014). In addition, genetic factors may influence the presence of haemorrhoidal disease. According to research published in 2020, there are a total of 102 risk factors for hemorrhoids in the human genome. This research suggests that hemorrhoids are influenced by genetics (Zheng et al., 2021).

Given the substantial prevalence of hemorrhoids and the potential for discomfort, pain, and bleeding (Mott et al., 2018), the urgency to advance effective treatments becomes paramount. Present strategies encompass adjustments in lifestyle, such as heightened dietary fiber intake, as well as utilization of oral medications and topical interventions like creams and ointments (Lohsiriwat 2012; Sun and Migaly, 2016). However, the efficacy of these measures might be limited, offering only transient relief. Consequently, the quest for innovative and potent solutions for hemorrhoid management is keenly anticipated. The integration of genetic markers represents a pioneering leap in the realm of hemorrhoid therapeutic exploration. Exploiting these genetic markers provides us with the means to unravel the intricate biological mechanisms underpinning hemorrhoids and precisely identify drug targets. In contrast to the predominantly time-intensive and costly trial-and-error approach characteristic of many drug discovery and development endeavors, a more systematic and rational methodology can be embraced to circumvent these challenges. One avenue that shows great promise involves harnessing the power of bioinformatics to forecast potential targets and novel drug candidates for hemorrhoid treatment. A comprehensive depiction of the study workflow is illustrated in Fig. 1, offering a detailed overview of the sequential steps undertaken in this innovative research pursuit.

2. Methods

2.1. Fetching hemorrhoid-associated genes

We conducted an extensive inquiry utilizing three authoritative databases renowned for their comprehensive genetic and disease-related data, accessed on July 12, 2023, to systematically assemble a comprehensive repertoire of genes implicated in the pathogenesis of hemorrhoids. These databases encompassed the GWAS Catalog (https://www.ebi.ac.uk/gwas/home) (Sollis et al., 2023), the Comparative Toxicogenomics Database (https://ctdbase.org/) (Davis et al., 2023), and the Open Targets Platform (https://www.targetvalidation.org/) (Ochoa et al., 2023). Our search methodology entailed querying these repositories using the term “hemorrhoids” to extract pertinent genetic insights. A stringent filtering threshold was applied for the Open Targets Platform, exclusively retaining genes with score values surpassing 0.3. This rigorous criterion ensured the inclusion of genetic information with heightened relevance and confidence. Following the meticulous implementation of these filtration parameters, the final compendium of hemorrhoid-associated genes was curated by eliminating duplicative entries. This amalgamation is a valuable and reliable resource for subsequent explorations and investigations into the genetic underpinnings of hemorrhoids and its associated disorders.

2.2. Prioritizing biological risk genes in hemorrhoid susceptibility

Applying a scoring system, five distinct functional annotations were integrated into the filter for genes associated with hemorrhoids. The criteria were as follows: (1) Knockout mouse phenotype (KOMP) - Gene contribution to specific mouse phenotypic diseases was assessed through Mammalian Phenotype Ontology (MP) from WebGestalt (2019), considering a False Discovery Rate (FDR) q-value < 0.05 as significant (Liao et al., 2019); (2) Kyoto Encyclopedia of Genes and Genomes (KEGG) - Molecular pathways were identified using KEGG (Kanehisa and Goto 2000) within WebGestalt, with a significance threshold set at q-value < 0.05 (Liao et al., 2019); (3) Gene Ontology (GO) – Utilizing biological process (BP), cellular component (CC), and molecular function (MF) categories, distinct biological functions related to hemorrhoids were identified. A GO enrichment analysis from WebGestalt was

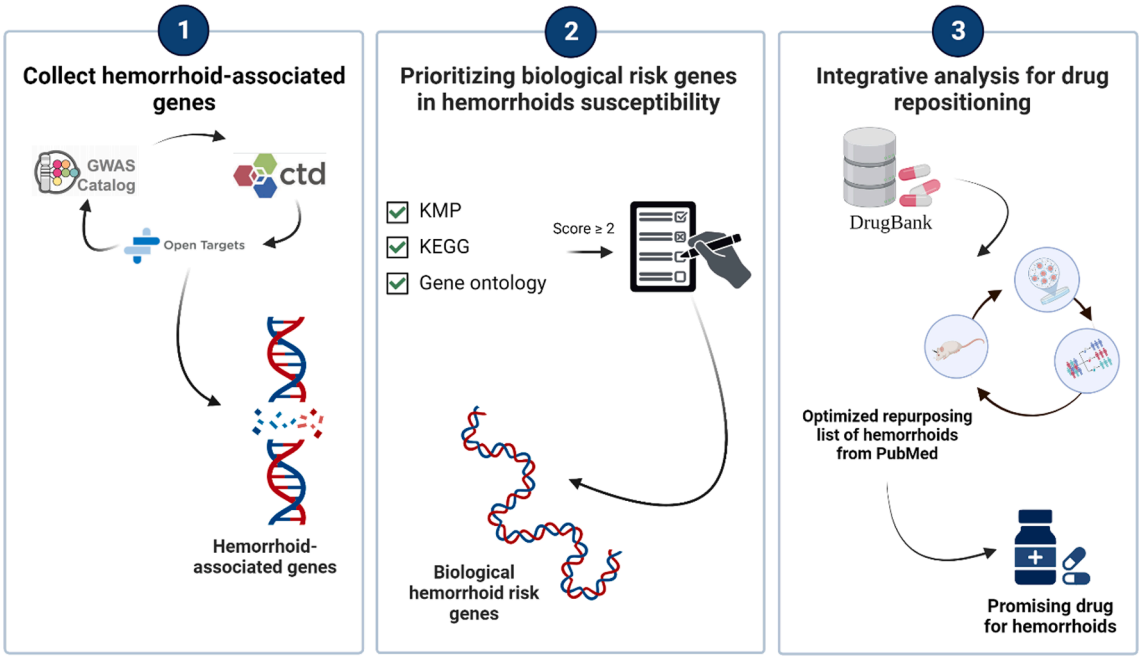

Fig. 1. An illustration of the bioinformatics-guided drug repositioning process for hemorrhoids. This figure was created with BioRender.com.

conducted, with a significance threshold of $q < 0.05$ (Liao et al., 2019). Genes scoring greater than or equal to 2 were classified as biological hemorrhoid-risk genes. Higher annotation scores corresponded to genes exerting a more pronounced biological impact on hemorrhoid pathogenesis, termed as "biological risk genes."

2.3. Mapping drug targets for hemorrhoids using DrugBank

During this phase, we employed DrugBank (data released on January 4, 2023) to associate biological risk genes linked to hemorrhoids with potential candidate drugs. DrugBank serves as an online repository offering comprehensive insights into drugs and their corresponding gene targets, serving as a valuable resource for bioinformatics and cheminformatics applications in drug discovery across clinical medical domains. Notably, DrugBank supports *in silico* drug target identification, drug design, docking or screening, prediction of drug metabolism and interactions, and facilitates general pharmaceutical education (Wishart et al., 2018). A range of parameters guided our database queries, including drugs demonstrating pharmacological activity, proven human efficacy, approved annotations, involvement in clinical trials, or designation as experimental agents. Additionally, each identified drug underwent verification on ClinicalTrials.gov (https://clinicaltrials.gov/; accessed on July 12, 2023) to ascertain its ongoing clinical investigation status for hemorrhoids or alternative medical conditions.

2.4. Statistical analyses

Statistical analyses were carried out using R (version 4.2.1). Over-representation analysis (ORA), encompassing KOMP, GO, and KEGG pathway enrichment analysis, was conducted utilizing the WebGestalt 2019 R package (Liao et al., 2019). Visualization of KOMP, GO, and KEGG results was accomplished using the ggplot2 package (R v4.2.1) (Wickham 2009). The Venn diagram, illustrating the intersection of five functional annotations, was generated using the dedicated R package (R v4.2.1) (Chen and Boutros 2011). Moreover, a chord diagram generated by the circlize package in R (Gu et al., 2014) and an alluvial diagram generated by the RAWGraphs visualization program were used to visualize the hemorrhoid candidates drugs (Mauri et al., 2017).

3. Results

3.1. Gene identification associated with hemorrhoids

We meticulously gathered genes linked to hemorrhoids by systematically extracting data from three prominent genomic databases: GWAS Catalog, Comparative Toxicogenomics Database, and the Open Targets Platform. These databases are renowned for their extensive genetic and disease-related information, making them ideal sources for our investigation. Through precise queries employing hemorrhoid-specific search terms, we retrieved a diverse array of genetic details pertinent to hemorrhoidal conditions. Following a rigorous process of data curation and the elimination of redundancies, we established a comprehensive compilation of 293 genes that are associated with hemorrhoids. These carefully documented genes are meticulously cataloged in **Table S1**, furnishing researchers with a valuable and meticulously curated resource. This compilation serves as an indispensable foundation for in-depth explorations and inquiries into the genetic intricacies of hemorrhoids and its correlated maladies. This curated gene assemblage not only aids in unraveling the molecular underpinnings of hemorrhoids but also holds the potential to uncover novel therapeutic targets. Furthermore, it contributes significantly to enhancing our comprehension of the genetic factors that influence the initiation and progression of hemorrhoid-associated conditions.

3.2. Prioritization of genes through functional annotation

We meticulously executed a rigorous functional annotation process to identify genes associated with the biological risk of hemorrhoids. To prioritize genes for potential drug discovery within the confines of this study, we adopted a scoring system previously employed and documented in various research publications (Irham et al., 2020; Adikusuma et al., 2021; Adikusuma et al., 2022; Afief et al., 2022; Zazuli et al., 2022; Adikusuma et al., 2023). The ensuing description presents the outcomes of the scoring process across five functional annotations: 1) genes prioritized via KOMP (n = 22); 2) genes prioritized through KEGG (n = 22); 3) genes prioritized based on BP (n = 67); 4) genes prioritized based on CC (n = 24); and 5) genes prioritized based on MF (n = 33). Comprehensive visual representations of the score distribution for each criterion are depicted in Fig. 2**A–C and Table S2**. Ultimately, a total of 38 genes, exemplifying characteristics indicative of a biological hemorrhoid risk, met the established criteria by attaining scores of 2 or higher. A more thorough examination of the gene scores revealed the preeminent status of two genes, *NOX1* and *NOS3*, both of which surpassed a score of 3, as elucidated in Fig. 2**A**. This comprehensive approach to gene prioritization scores not only facilitates the identification of pivotal candidate genes for subsequent investigation but also unveils notable targets, such as *NOX1* and *NOS3*, which hold considerable promise within the realm of hemorrhoid research.

Moreover, the outcomes stemming from the five distinct functional annotations encapsulate the realms of KOMP, KEGG, BP, CC, and MF. Notably, the KEGG analysis strategically identified the major pathway as "Fluid shear stress and atherosclerosis," furnishing a pivotal molecular context that underscores prospective mechanistic associations between hemorrhoids and this specific pathway. Delving into the KOMP domain, the findings intriguingly underscored a pronounced correlation with "abnormal wound healing." This revelation implies a nuanced connection between the identified genetic factors and the intricate processes of tissue healing that could play a role in hemorrhoidal development. In the sphere of BP, a pervasive engagement was noted in the "regulation of cell death," unveiling a central biological facet that likely contributes to the intricate interplay of genetic elements and the manifestation of hemorrhoids. Simultaneously, the MF analysis cast light on a pivotal molecular mechanism, specifically "cofactor binding," signaling potential interactions that play an integral role in the underlying biological processes associated with hemorrhoids. Furthermore, the CC exploration underscored the prominence of the "plasma membrane protein complex," highlighting the significance of this specific cellular structure in the context of gene associations related to hemorrhoids.

To visually encapsulate the significance of the KOMP, KEGG, and GO enrichment analyses (BP, MF, CC), we present Fig. 3**A–E**. This figure functions as a succinct summary, vividly illustrating the enriched functions within each category. It accentuates the pivotal KOMP, KEGG pathways, key biological processes, cellular components, and molecular functions interwoven with the identified genes, offering an inclusive snapshot of their functional roles. Detailed results for all five functional annotations are comprehensively outlined in **Table S3**, thereby serving as a comprehensive resource for further exploration and investigation.

3.3. Comprehensive analysis for drug repositioning

To discern potential therapeutic candidates for hemorrhoids, we embarked on a process of aligning genes associated with biological hemorrhoid risk to relevant drugs within the DrugBank database. This investigative approach sought to ascertain whether genes culled from the profiles of biologically risky genes could serve as pharmacologically viable targets for approved drugs. This comprehensive endeavor culminated in the identification of a total of 87 drugs, all of which exhibited targeting interactions with 21 distinct hemorrhoid risk genes. Consequently, these drugs hold significant promise as prospective candidates for the therapeutic management of hemorrhoids, as meticulously

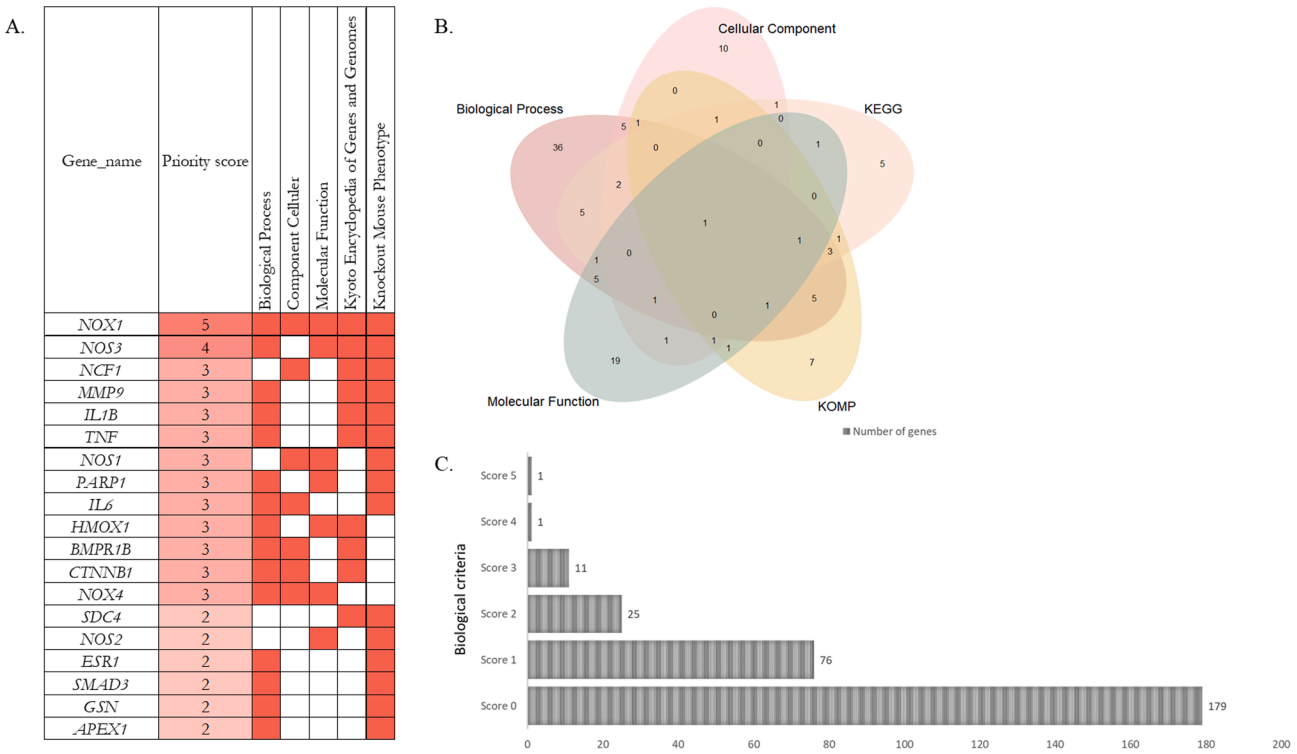

Fig. 2. Systematic prioritization of candidate genes from hemorrhoid risk loci based on multi-criteria analysis. (A) Summary scores based on five criteria are displayed. Fulfilled criteria are represented by filled boxes. Genes with a score of ≥2 were identified as "biological hemorrhoid risk genes." Refer to **Table S2** for comprehensive information. (B) The Venn diagram presents the prioritization criteria used to select the biological candidate gene from the hemorrhoid risk loci. The distribution of gene scores is presented in the histogram. The figure displays 38 genes that have a total score of ≥2.

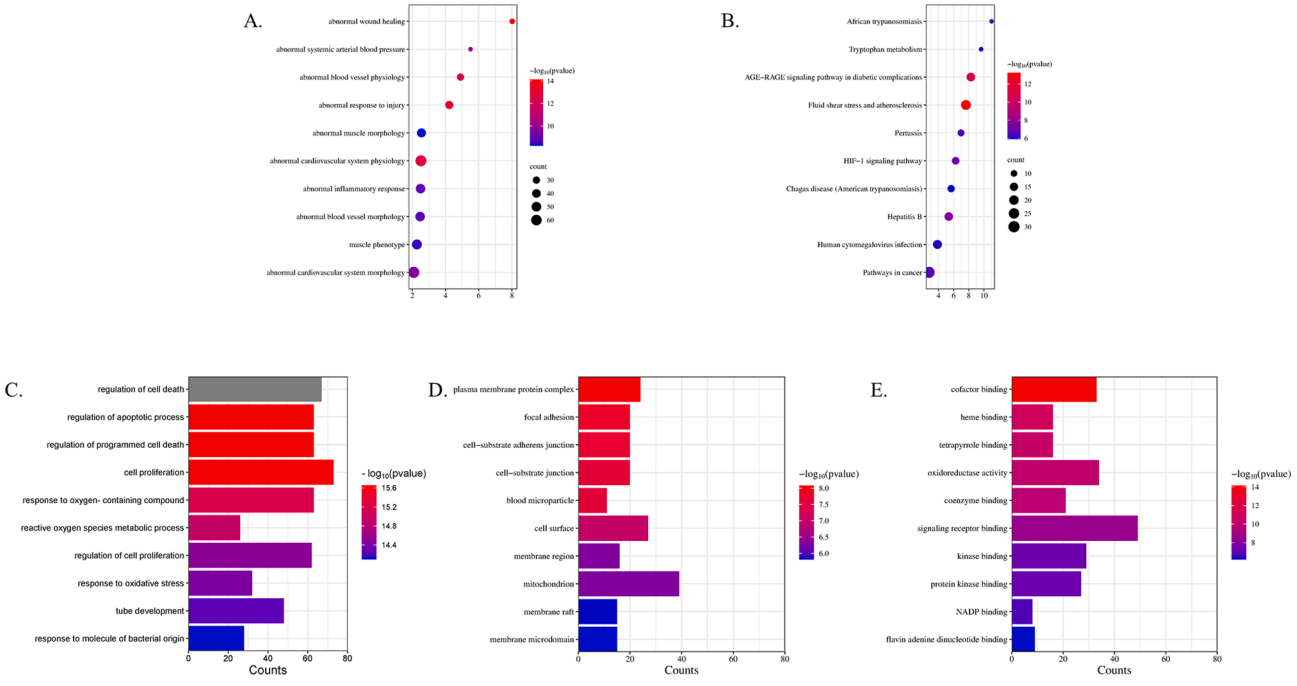

Fig. 3. The top 10 enriched analyses for five functional annotations were identified as follows: (A) Knockout mouse phenotype, (B) Kyoto encyclopedia of genes and genomes, (C) Biological process, (D) Cellular component, (E) Molecular function.

detailed in **Table S4**.

Within this range of 87 drugs, a diverse landscape emerged: ketamine (NCT04248205) was under active clinical investigation for hemorrhoids, fulvestrant was supported by preclinical in vitro models demonstrating potential efficacy for hemorrhoids (Fanyu Meng et al., 2022), and methylene blue was supported by case series evidence for hemorrhoids (Baldiwala and Trivedi, 2022; Long et al., 2023), as shown in Fig. 4. Notably, 84 of these compounds represented novel therapeutic avenues not previously explored in the treatment of hemorrhoids (Fig. 5). A particularly noteworthy discovery within our investigation

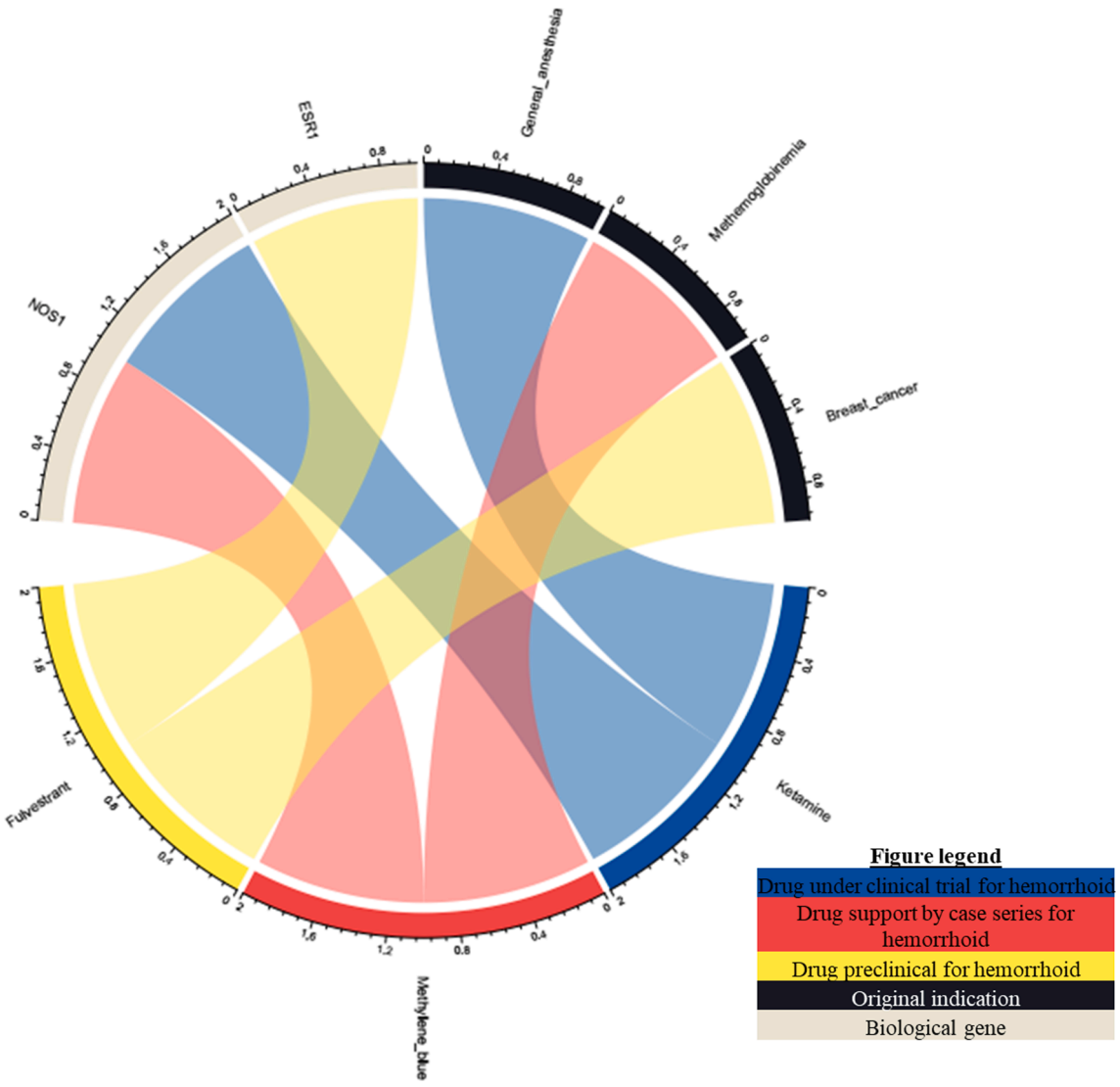

Fig. 4. Biological links between hemorrhoid risk genes and drugs potentially useful for hemorrhoids.

was the identification of *NOS3* overlapping sapropterin, which surfaced as an exceptionally promising target for hemorrhoid intervention. This assessment was further fortified by its attainment of a high systemic score in functional annotations, affirming its potential relevance as a critical molecular player in hemorrhoidal pathogenesis. Collectively, our study serves as a compelling proof-of-principle, illustrating that genomic data not only facilitates the discovery of potential biomarkers for hemorrhoids but also propels the realm of drug repurposing for this debilitating affliction. This innovative synergy of genomic insights and drug repurposing underscores a transformative paradigm that holds the potential to alleviate the burden of this ailment through targeted therapeutic interventions.

4. Discussion

Advancements in genomic research have improved our understanding of the pathogenic mechanisms present in a variety of disorders, including hemorrhoids. Genetic research aims to uncover the biological complexities of diseases, instead of merely identifying susceptibility genes, and then translating these findings into practical clinical applications (Green et al., 2011). Although several genetic risk loci associated with various human phenotypes have been identified, the merging of genetic research insights into biologically relevant risk genes has been inadequately explored (Green et al., 2011; Webb 2012). Our study seeks to predict therapeutic targets and innovative drug candidates for the treatment of hemorrhoidal afflictions. Our methodology employs genomic networks and advanced bioinformatics analyses, leading to the development of detailed genomic networks that uncover the complex molecular interactions involved in disease progression. These networks incorporate hemorrhoid-associated genes from diverse databases, providing valuable understanding of the genetic foundation of hemorrhoids, and proposing encouraging implications for the detection of feasible diagnostic biomarkers and improved therapeutic approaches. The detection of novel biomarkers is critical for classifying patients, foretelling therapeutic responses, and evaluating prognosis (Santri et al., 2022).

In our study identified 87 drugs that target 21 genes, of which 3 have clinical and preclinical validation, demonstrating the effectiveness of our approach in guiding hemorrhoid treatment. Therefore, our findings emphasize the value of implementing a combined approach that integrates genomic networks and bioinformatics analyses, providing a robust scientific foundation to advance drug discovery efforts for hemorrhoids. Among the drugs identified, three have received support from clinical and preclinical evidence for hemorrhoids. Ketamine, having completed phase IV clinical trials (NCT04248205), operates as an N-methyl-D-aspartate (NMDA) receptor antagonist and exhibits

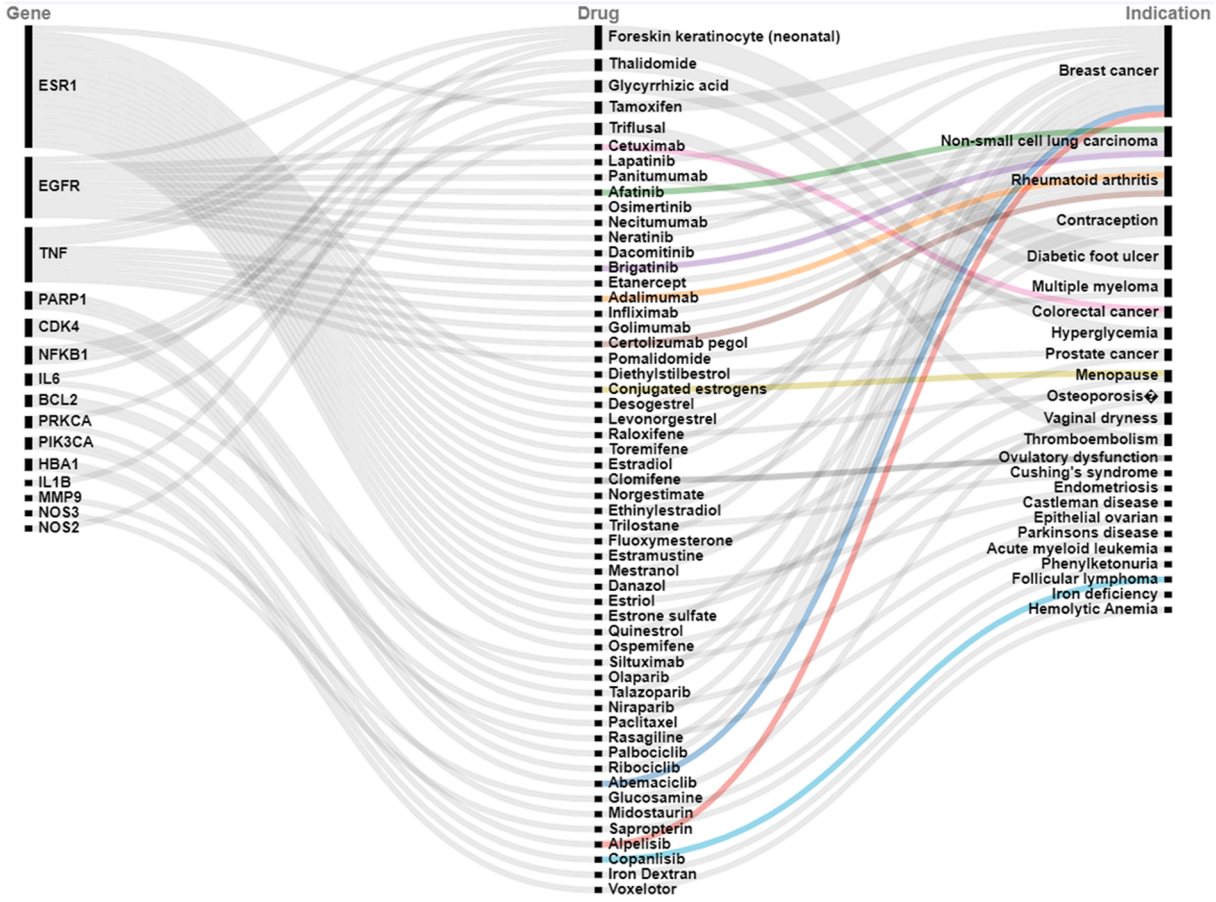

Fig. 5. Associations between biological risk genes for hemorrhoid and drugs that have been approved for other indications. For complete information, see **Table S4**.

effectiveness in reducing sensitization and postoperative pain. It offers superior analgesia compared to placebo without affecting cognitive function, hence relieving post-hemorrhoidectomy pain. Fulvestrant, an estrogen receptor antagonist, has shown promise in preclinical in vitro models by inhibiting ERα, thereby reducing VEGF expression and mitigating postoperative hemorrhoid edema (Fanyu Meng et al., 2022). Methylene blue, supported by case series evidence, demonstrates anti-inflammatory effects by suppressing nitric oxide production, ultimately alleviating post-hemorrhoidectomy pain (Baldiwala and Trivedi, 2022; Long et al., 2023).

The investigation has identified the two most promising biomarkers among the biological hemorrhoid risk genes: These are NADPH oxidase 1 (*NOX1*) and nitric oxide synthase 3 (*NOS3*). *NOX1* has a pivotal role in generating reactive oxygen species (ROS), which are involved in the pathogenesis of numerous diseases, such as cancer, constipation, diabetes, neurodegenerative disorders, and inflammatory bowel disease (Valko et al., 2007, Schwerd et al., 2018; Hauck et al., 2019; Attri et al., 2020; Wei et al., 2022). However, the involvement of *NOX1* in the pathogenesis of hemorrhoids has not been explicitly explained in the current literature. According to our predictive analysis, *NOX1* is identified as a strong candidate for potential biomarker status in the context of hemorrhoids. The close interplay between *NOX1*, inflammation, and oxidative stress within the haemorrhoidal milieu highlights the possibility that *NOX1* levels or activity could be indicative biomarkers. Oxidative stress induced by ROS plays a significant role in initiating and progressing various pathological conditions, including hemorrhoids (Kusumawati et al., 2022). Increased *NOX1* expression or higher ROS production could indicate the level of inflammation and oxidative stress in tissues affected by hemorrhoids. Therefore, evaluating *NOX1* levels dynamically might provide a better understanding of the severity of hemorrhoids, facilitating informed treatment decisions.

Distinct protein expression patterns, with significantly higher levels of *NOS3* and *NOS1* in hemorrhoidal tissues compared to rectal tissue (Lohsiriwat et al., 2020), suggest *NOS3* as a potential important biomarker for hemorrhoids. The elevated immunoreactivity of *NOS3* in the vascular endothelium of hemorrhoids indicates exposure of these blood vessels to increased levels of nitric oxide (NO), which is known for its vasodilatory effects. The clinical significance of these results suggests potential therapeutic implications for the management of hemorrhoids (Lohsiriwat et al., 2020). Considering the notable vascular dilation, bleeding, and swelling associated with the condition, regulating *NOS3* activity provides an interesting avenue for intervention. Using NOS inhibitors to decrease NO production could provide a targeted approach to reduce the exaggerated vasodilation and relieve the characteristic symptoms of hemorrhoids (Gokce et al., 2020).

Notably, among the top two potential biomarkers identified for hemorrhoids, it is essential to highlight that *NOS3* stands out as a druggable target, offering a unique avenue for therapeutic intervention. What sets *NOS3* apart is its alignment with an existing drug, sapropterin, presenting an intriguing opportunity for innovative hemorrhoid therapy. Sapropterin's primary indication lies in the management of hyperphenylalaninaemia (HPA) among patients afflicted with phenylketonuria (PKU) and/or tetrahydrobiopterin (BH4) deficiency, demonstrating remarkable responsiveness to this treatment regimen (Sanford and Keating 2009; Dubois and Cohen 2010). The proven effectiveness of sapropterin in modulating these physiological processes raises the prospect of its applicability to other conditions characterized by dysregulated NO synthesis, such as hemorrhoids. Given that *NOS3* is implicated in the regulation of NO production, and NO itself plays a critical role in vascular dynamics and tissue responses (Lohsiriwat et al., 2020; Gokce et al., 2020), sapropterin's modulation of *NOS3* activity could have profound implications for hemorrhoid therapy. By fine-tuning

NOS3-mediated NO synthesis, sapropterin holds the potential to exert a favorable influence on vascular tone, inflammation, and tissue repair within the hemorrhoidal microenvironment. However, while the rationale for repurposing sapropterin for hemorrhoid therapy appears promising, rigorous research is imperative to validate its efficacy, safety, and optimal dosing in this novel context. Clinical investigations exploring sapropterin's impact on *NOS3* activity within hemorrhoidal tissues, its potential to ameliorate vasodilation, and its overall influence on symptom relief are vital steps toward unlocking its therapeutic potential for hemorrhoids. The intriguing convergence of *NOS3*, sapropterin, and hemorrhoids invites further exploration and underscores the importance of innovative drug repurposing in advancing hemorrhoid treatment options.

Moreover, a notable study limitation is the potential introduction of biases in both the DrugBank database and the construction of genomic networks. Our study heavily depends on DrugBank for critical drug-gene associations and pharmacological data. Recognizing that curated databases like DrugBank may introduce biases is essential. The data within DrugBank could incline towards extensively researched or clinically significant drugs, potentially affecting a comprehensive representation of all existing drug-gene associations. Biases in data curation, updates, and drug inclusion criteria within DrugBank can impact the accuracy and completeness of utilized drug-gene associations. Furthermore, constructing genomic networks entails integrating data from various sources, encompassing databases and experimental studies. Acknowledging limitations such as data quality, heterogeneity, and variations in experimental methodologies is crucial as they may introduce biases in the resulting network. The topology and inferred gene-gene interactions in the network could be influenced by the chosen construction methods.

5. Conclusion

Integrated genomic network analysis highlighted 38 genes as biological hemorrhoid-risk genes, with *NOX1* and *NOS3* being the most significant. Notably, *NOS3* is a druggable target and one of the top two potential biomarkers identified for hemorrhoids, providing a unique avenue for therapeutic intervention. Its alignment with sapropterin, an existing drug that presents an intriguing opportunity for innovative hemorrhoid therapy. In conclusion, this study underscores the potential and practicality of integrated genomic network analysis in driving drug discovery for hemorrhoids. Future research should focus on validating the therapeutic efficacy of sapropterin targeting NOS3 and explore other potential druggable targets identified through this approach to advance hemorrhoid treatment strategies. Taken together, our study provided insights into hemorrhoid management genomic profiles in clinical settings, offering further insight into genomic-based therapies and drug discovery.

CRediT authorship contribution statement

Wirawan Adikusuma: Data curation, Formal analysis, Writing – original draft. **Firdayani Firdayani:** Data curation, Formal analysis. **Lalu Muhammad Irham:** Data curation, Formal analysis. **Darmawi Darmawi:** Data curation. **Muhammad Yulis Hamidy:** Data curation. **Baiq Leny Nopitasari:** Data curation. **Soraya Soraya:** Data curation. **Nurul Azizah:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by grants from Ministry of Education, Culture, Research, and Technology of Indonesia, Indonesia. (No. 3547/LL8/AL.04/2023).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101831.

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