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Research Article

Integrative genomic analysis of missense mutations and target genes in hemorrhoid pathogenesis

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Abstract

Introduction: Hemorrhoids are a prevalent anorectal condition characterized by the enlargement and displacement of anal cushions. While environmental and lifestyle factors are well-established contributors, emerging evidence suggests a genetic predisposition involving connective tissue integrity and smooth muscle dysfunction. However, the specific genetic variants contributing to hemorrhoid pathogenesis remain largely undefined.

Aim: This study aimed to identify and characterize potentially pathogenic single-nucleotide polymorphisms (SNPs) associated with hemorrhoids to better understand their molecular and functional implications.

Materials and methods: SNPs associated with hemorrhoids were retrieved from the Genome-Wide Association Studies (GWAS) Catalog. Functional annotations and pathogenicity predictions were performed using PolyPhen-2, SIFT, SNPnexus, and GTEx to assess structural and regulatory impacts of the variants on gene function and expression in relevant tissues.

Results: Functional annotation of hemorrhoid (HEM)-associated SNPs revealed that rs2186797 (*ANO1*) and rs35318931 (*SRPX*) may impact protein structure and function, as predicted by PolyPhen-2 (possibly damaging). In contrast, rs8176746 (*ABO*) was predicted to be benign. SIFT analysis identified rs2186797 as deleterious across multiple amino acid positions, indicating potential disruption of *ANO1* function. Pathway enrichment analysis linked rs2186797 to key biological processes, including stimuli-sensing channels and ion channel transport, suggesting a role for *ANO1* in ion transport mechanisms relevant to HEM pathogenesis.

Conclusions: These findings highlight the potential functional impact of *ANO1*-related genetic variation in HEM and underscore its involvement in ion transport pathways, providing insight into the molecular mechanisms underlying HEM pathogenesis.

Keywords

bioinformatics, GWAS, genomic variants, missense mutations

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PENSOFT.

Introduction

Hemorrhoids are normal vascular cushions located at the junction of the rectum and anus, playing a presumed role in maintaining continence through their contribution to the anal sealing mechanism.[1,2] Owing to their rich sensory innervation, they are also believed to be involved in sensing fullness, pressure, and the nature of anal contents.[3,4] Hemorrhoidal disease (HEM) arises when these cushions become enlarged and symptomatic, commonly presenting with rectal bleeding, pruritus, and soiling. These symptoms often result from prolapse, connective tissue degeneration, venous dilation, or thrombosis. Severe cases frequently require surgical intervention, including the excision of thrombosed or prolapsed HEM.[2]

HEM is highly prevalent worldwide, with estimates suggesting up to 4.4% of individuals may experience symptoms during their lifetime.[5] However, many cases remain undiagnosed due to mild or asymptomatic presentation. The condition imposes a considerable medical and socioeconomic burden, with annual healthcare costs in the United States alone exceeding USD 800 million, primarily due to surgical procedures.[6] Multiple risk factors have been proposed, including the human erect posture, hypothesized to have co-evolved with the complex hemorrhoidal plexus, as well as sedentary behavior, obesity, low fiber intake, prolonged straining, constipation, diarrhea, pelvic floor dysfunction, and childbirth.[7] However, the molecular mechanisms underlying HEM remain poorly understood, and it is unclear why only some individuals develop clinically significant disease. Emerging evidence suggests that genetic predisposition may play a crucial role in HEM pathogenesis. A recent genome-wide study identified over 100 genetic loci associated with increased risk for HEM, underscoring the importance of host genetic factors.[8]

Aim

To elucidate the genetic underpinnings of HEM by conducting an integrative genomic analysis focusing on missense mutations and their corresponding target genes. By harnessing publicly available GWAS data across multiple populations and applying a suite of bioinformatics tools, we identify key variants that may influence disease development. Our workflow, summarized in **Fig. 1**, combines variant prioritization, functional annotation, population frequency analysis, and tissue-specific gene expression profiling. This approach not only enhances our understanding of HEM genetics but also lays the foundation for future diagnostic, preventive, and therapeutic strategies tailored to individual genetic risk profiles.

Materials and methods

Acquisition of GWAS datasets involved HEM-associated SNPs

The GWAS Catalog database maintained by the National Human Genome Research Institute (NHGRI) (https://www.ebi.ac.uk/gwas/efotraits/EFO_0009552) was queried to identify single-nucleotide polymorphisms (SNPs) associated with HEM.[9] Using the trait identifier "hemorrhoid" (EFO_0009552), all available association records were downloaded on May 30, 2025. This search yielded 107 risk-associated variants reported across 38 publications. The dataset was then filtered to retain only missense variants with genome-wide significance ($p<1\times10^{-8}$), resulting in the identification of three missense mutations.

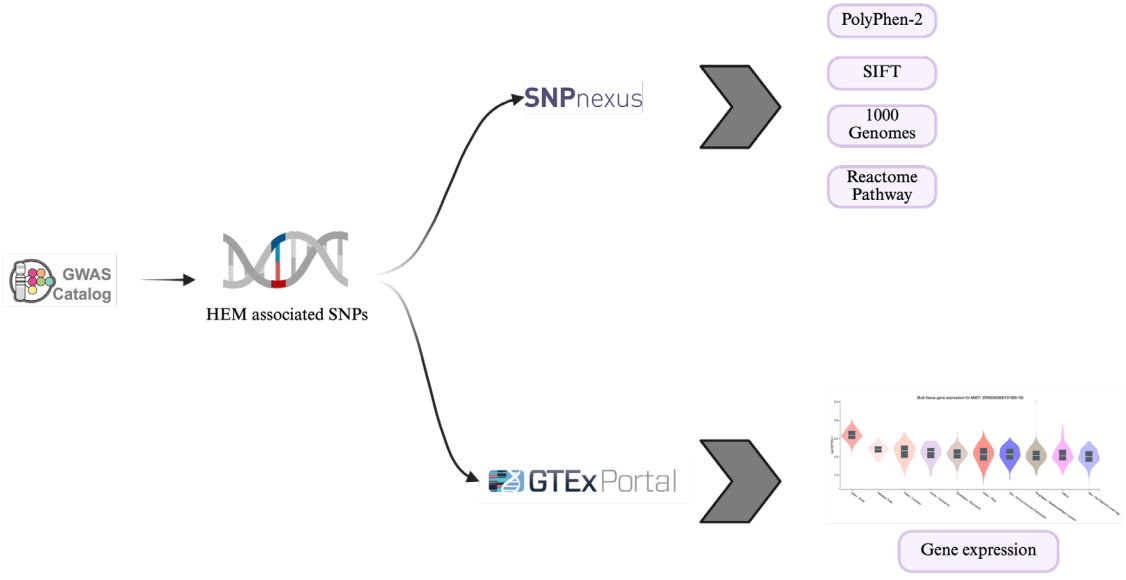

Figure 1. Data processing scheme from the genomic database using bioinformatics for genomic variant identification.

Impact of non-synonymous SNPs on protein function

Following data filtering and removal of duplicate entries based on rsID and chromosomal position using Microsoft Excel, three unique missense SNPs were retained for further analysis. These variants were submitted to SNPnexus (https://www.snp-nexus.org/v4/) for functional annotation, specifically using the integrated PolyPhen-2 and SIFT algorithms to assess their potential impact on protein structure and function. PolyPhen-2 predicts the functional significance of amino acid substitutions based on sequence conservation, structural features, and known protein domains. It classifies variants into three categories: benign (score 0.0–0.49), possibly damaging (0.5–0.89), and probably damaging (0.9–1.0).[10] These thresholds were used to interpret all PolyPhen-2 scores reported in this study.

To complement this structural evaluation, the SIFT algorithm was applied to evaluates the impact of amino acid substitutions on protein function by analyzing sequence homology and the physicochemical properties of residues. It assesses the evolutionary conservation of amino acids across species to estimate the likelihood that a substitution will affect protein activity. Variants classified as "intolerant" are more likely to be functionally disruptive, while "tolerant" variants are typically considered neutral.[11,12] Together with PolyPhen-2, SIFT enables comprehensive functional annotation of missense variants, supporting the interpretation of their potential biological significance and contribution to disease processes.

Assessing population allele frequencies with 1000 genomes data

SNPnexus was also employed to determine the population-specific statistics of the identified SNPs using data from the 1000 Genomes Project. Population groups were categorized into five major continental regions: Africa, the Americas, Europe, East Asia, and South Asia. This database provides a comprehensive reference for human genetic variation, enabling the estimation of allele frequencies across diverse populations and supporting the assessment of geographic distribution and potential population-specific risk patterns.[13]

Mapping gene expression across tissues

Following PolyPhen analysis, the three genes corresponding to the identified SNPs were further examined for tissue-specific expression using the GTEx Portal (https://www.gtexportal.org/home/), with data extracted on May 30, 2025. The Genotype-Tissue Expression (GTEx) project is a comprehensive resource designed to explore the complexity of genetic variation and gene regulation across multiple human tissues. It facilitates the interpretation of GWAS findings in the context of functional genomics and translational research. In this study, bulk tissue gene expression levels were assessed, and the top ten tissues were selected based on expression level (log-transformed median values).[14]

Pathway enrichment analysis

SNP annotation and pathway enrichment analysis were conducted using SNPnexus (https://www.snp-nexus.org/). The tool was used to functionally annotate submitted SNPs by integrating data from multiple biological databases. Specifically, SNPnexus mapped each variant to genes and corresponding pathways using the Reactome pathway database.[10,15] Enrichment analysis was performed to identify pathways significantly associated with the genes containing the queried variants. Statistical significance for pathway associations was calculated using the hypergeometric distribution, and *p*-values were generated accordingly.

Results

Missense variant profiles in *ABO*, *SRPX*, and *ANO1* genes

The three identified SNPs are predicted to contribute to the pathogenesis of HEM by modulating protein function. As shown in **Table 1**, these missense variants were extracted from the GWAS Catalog database and are associated with HEM. Missense variants result in amino acid substitutions within proteins, which can alter their structure and function. Pathogenic missense mutations may disrupt normal protein activity and contribute to disease phenotypes, while benign variants are generally functionally neutral. Therefore, it is essential to focus on missense mutations that are likely to impact protein function. To ensure the validity of the associations, a stringent genome-wide significance threshold (p-value $<1\times10^{-8}$) was applied. This threshold, commonly used in GWAS, minimizes the likelihood of false positives that may arise from multiple hypothesis testing and enhances the robustness and reproducibility of the findings.[9]

Table 1. Missense variants of HEM associated with SNPs with p-value $<10^{-8}$

SNPs	P-value
rs2186797	4×10^{-8}
rs8176746	2×10^{-15}

Furthermore, **Table 2** summarizes the predicted functional impact of selected missense SNPs associated with HEM, analyzed using PolyPhen-2 and SIFT. To evaluate the potential functional consequences of missense variants associated with HEM, we performed in silico analyses using PolyPhen-2 and SIFT. PolyPhen-2 predicted that

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Table 2. Predicted functional impact of genetic variants associated with HEM-related SNPs on protein structure and function based on PolyPhen-2 analysis

SNPs	Chromosome	Variant	Gene	AA Position	Score	Prediction
rs2186797	chr11	T/C	*ANO1*	550	0.1	Benign
rs2186797	chr11	T/C	*ANO1*	608	0.58	Possibly damaging
rs2186797	chr11	T/C	*ANO1*	462	0.137	Benign
rs2186797	chr11	T/C	*ANO1*	317	0.217	Benign
rs8176746	chr9	G/T	*ABO*	265	0.003	Benign
rs35318931	chrX	G/A	*SRPX*	413	0.694	Possibly damaging
rs35318931	chrX	G/A	*SRPX*	354	0.83	Possibly damaging
rs35318931	chrX	G/A	*SRPX*	393	0.568	Possibly damaging

SNPs: single nucleotide polymorphisms; chr: chromosome; variant: nucleotide substitution (reference/alternate); AA: amino acid position; score: PolyPhen-2 prediction score (0.0–1.0); prediction: PolyPhen-2 classification of variant impact.

the variant rs2186797 in the *ANO1* gene may have a variable impact depending on the amino acid position, ranging from benign (e.g., position 550, score=0.1) to possibly damaging (e.g., position 608, score=0.58). Similarly, the variant rs35318931 in *SRPX* was consistently classified as possibly damaging across multiple amino acid positions. In contrast, the rs8176746 variant in *ABO* was predicted as benign. It is worth noting that although variants in the *ABO* gene appear benign in this analysis, genetic variation in *ABO* has been implicated in several health conditions, including cardiovascular disease and pancreatic cancer.[16] Thus, while PolyPhen-2 predictions offer valuable insights, integrating these data with clinical findings is essential for a comprehensive understanding of variant pathogenicity.[17]

SIFT analysis **(Table 3)**, which emphasizes evolutionary conservation, consistently classified all observed *ANO1* variant positions (317, 462, 550, 608) associated with rs2186797 as deleterious (score=0), suggesting a potential impact on protein function. However, variants in *ABO* (rs8176746) and *SRPX* (rs35318931) were predominantly predicted to be tolerated, with SIFT scores ranging from 0.28 to 0.62. These differing predictions between SIFT and PolyPhen-2 underscore the importance of integrating multiple tools when interpreting the effects of missense variants.

Population distribution of hemorrhoid-associated pathogenic variants

Table 4 presents the allele frequencies of three selected pathogenic SNPs: rs2186797, rs8176746, and rs35318931, across five major population groups: African (AFR), American (AMR), East Asian (EAS), European (EUR), and South Asian (SAS), based on data from the 1000 Genomes Project. For each SNP, the reference (REF) and alternate (ALT) alleles are provided, along with their respective allele frequencies within each population. Notably, the variant rs8176746 (G>T) exhibits the highest allele frequency in the SAS population at 23.31%, whereas its frequency is lowest in the AMR population at 4.76%. Conversely, rs35318931 (G>A) is absent in the EAS population, indicating a potential population-specific absence or rarity, but

Table 3. Predicted functional impact of genetic variants associated with HEM-related SNPs on protein structure and function based on SIFT analysis

SNPs	Chromosome	Variant	Gene	AA Position	Score	Prediction
rs2186797	chr11	T/C	*ANO1*	550	0	Deleterious
rs2186797	chr11	T/C	*ANO1*	608	0	Deleterious
rs2186797	chr11	T/C	*ANO1*	462	0	Deleterious
rs2186797	chr11	T/C	*ANO1*	317	0	Deleterious
rs8176746	chr9	G/T	*ABO*	265	0.39	Tolerated
rs8176746	chr9	G/T	*ABO*	265	0.28	Tolerated
rs35318931	chrX	G/A	*SRPX*	413	0.6	Tolerated
rs35318931	chrX	G/A	*SRPX*	354	0.61	Tolerated
rs35318931	chrX	G/A	*SRPX*	393	0.62	Tolerated

SNPs: single nucleotide polymorphisms; chr: chromosome; Variant: nucleotide substitution (reference/alternate); AA: Amino Acid position; Score: SIFT prediction score (0.0–1.0); Prediction: SIFT classification (Deleterious or Tolerated)

Table 4. Allele frequencies of selected pathogenic variants across major populations

SNPs	REF allele	ALT allele	Allele global frequency				
			AFR	AMR	EAS	EUR	SAS
rs8176746	G	T	0.1694	0.0476	0.1935	0.0845	0.2331
rs35318931	G	A	0.005	0.0286	0.0000	0.0705	0.0097

SNPs: Single Nucleotide Polymorphisms; REF: Reference allele; ALT: Alternate allele; AFR: African population; AMR: American population; EAS: East Asian population; EUR: European population; SAS: South Asian population.

shows a higher frequency in the EUR population (7.05%). The variant rs2186797 (T>C) demonstrates its highest frequency in the EUR population (6.36%), underscoring regional differences in the distribution of these genetic variants. These data highlight the importance of considering population-specific allele frequencies in genetic association studies and suggest that the contribution of these SNPs to disease risk may vary across different ethnic groups.

Gene expression related to hemorrhoids and SNP

Tissue-specific expression of *ABO*, *SRPX*, and *ANO1* was analyzed using GTEx Portal data (**Figs 2, 3, 4**). *ABO* was highly expressed in the ovary and small intestine, but showed moderate to low expression in anorectal-relevant tissues like the colon and esophagus. *SRPX* was primarily expressed in fibroblasts and visceral adipose tissue—tissues involved in ECM remodeling, which may influence HEM pathology. *ANO1* displayed robust expression in vascular and smooth muscle tissues, including the aorta, coronary artery, and gastrointestinal tract. This pattern supports its functional involvement in smooth muscle regulation and epithelial ion transport, key processes in hemorrhoidal disease.

Pathway annotation and enrichment analysis via SNPnexus

Pathway enrichment analysis identified three pathways associated with rs2186797 in *ANO1*, all of which fall under the "Transport of small molecules" category **(Table 5)**.[10,15,18,19] The stimuli-sensing channels (p=0.0098) were the most important pathway, followed by the ion channel transport (p=0.0169) and transport of small molecules (p=0.0675). These pathways implicate *ANO1* in ion transport, sensory signaling, and epithelial secretion—all of which are relevant to the vascular tone and mucosal environment of hemorrhoidal tissue.[20,21] Together, these findings highlight *ANO1* as a key candidate gene with predicted deleterious missense mutations, functional pathway involvement, and strong tissue-specific expression in rele-

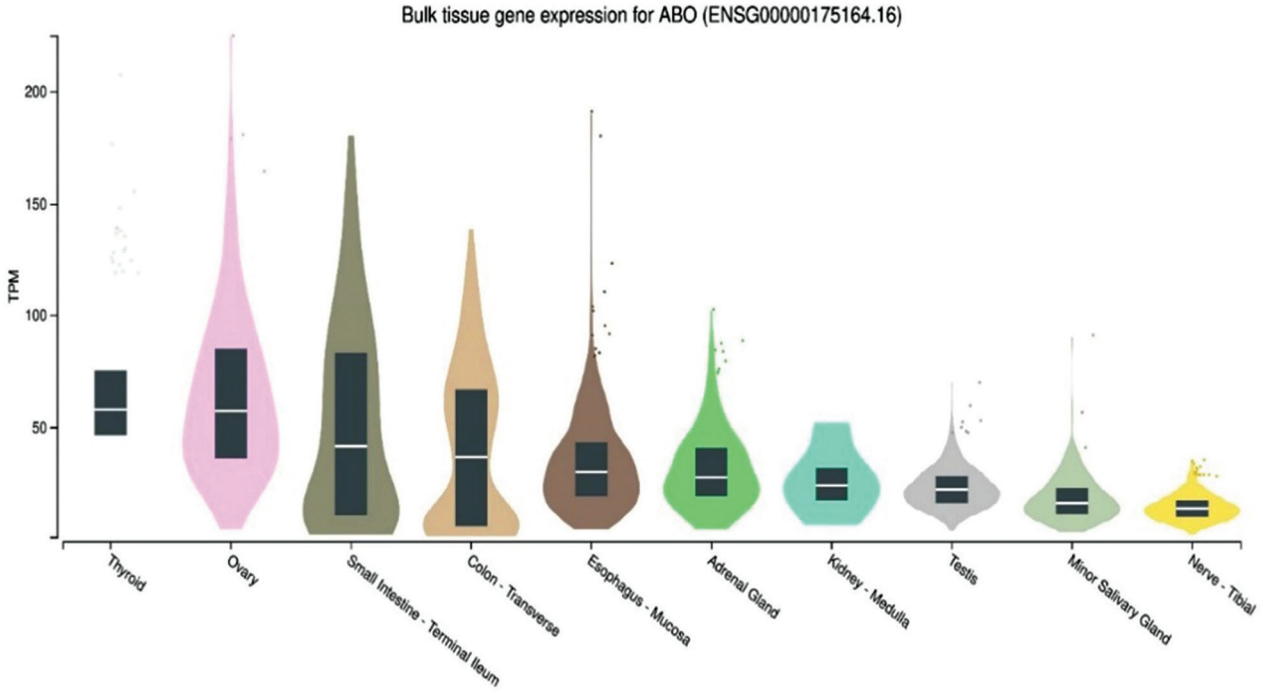

Figure 2. The ten most highly expressed ABO tissue genes based on the GTEx Portal.

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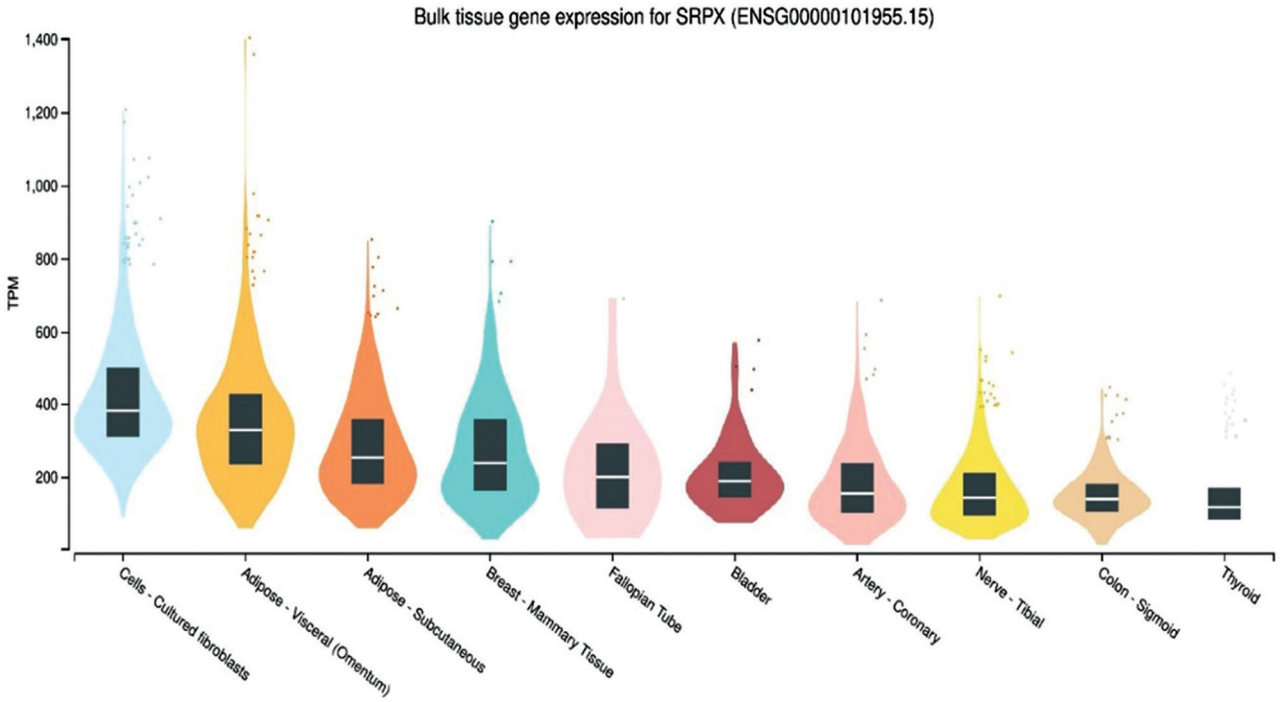

Figure 3. The ten most highly expressed SRPX tissue genes based on the GTEx Portal.

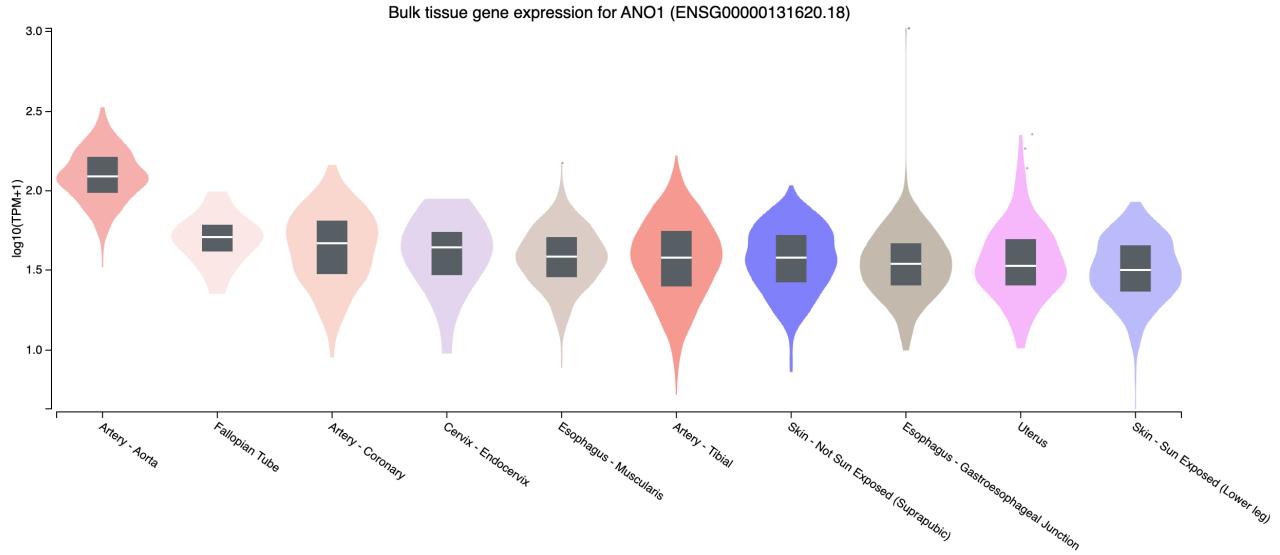

Figure 4. The ten most highly expressed ANO1 tissue genes based on the GTEx Portal.

Table 5. Pathway Enrichment Analysis involving *ANO1* and rs218679

Pathway ID	Description	Parent(s)	p-Value	Genes involved	Variation IDs
R-HSA-2672351	Stimuli-sensing channels	Transport of small molecules	0.009838	*ANO1*	rs2186797
R-HSA-382551	Transport of small molecules	Transport of small molecules	0.067564	*ANO1*	rs2186797
R-HSA-983712	Ion channel transport	Transport of small molecules	0.016984	*ANO1*	rs2186797

Pathway ID: Reactome pathway identifier; genes involved: Genes associated with the enriched pathway; Variation IDs: corresponding SNP identifiers; ANO1: Anoctamin-1 (calcium-activated chloride channel protein)

vant anatomical sites, reinforcing its role in HEM pathophysiology.

Discussion

Despite the widespread prevalence of HEM, the genetic and molecular underpinnings of its pathophysiology remain insufficiently characterized. In this study, we leveraged genome-wide association data and in silico bioinformatics tools to evaluate the potential impact of missense variants associated with HEM. Among the candidate genes analyzed, *ANO1* emerged as the most compelling, based on consistent predictions of functional disruption, gene expression patterns, and pathway relevance.

ANO1 (also known as *TMEM16A*) encodes a calcium-activated chloride channel (CaCC) that plays a pivotal role in regulating smooth muscle tone, epithelial ion transport, and sensory signaling—biological functions that are closely linked to anorectal physiology.[20-22] The rs2186797 variant in *ANO1* was predicted to be deleterious by SIFT (score=0 at all evaluated positions) and possibly damaging by PolyPhen-2 (score up to 0.58), suggesting a high probability of altered protein function. These predictions are reinforced by the gene's high expression in vascular and gastrointestinal tissues, particularly the aorta, esophagus, and arterial smooth muscle, as shown in GTEx data.[14] Given the smooth muscle-rich nature of hemorrhoidal cushions, *ANO1* dysfunction may contribute to venous dilation, altered contractility, and mucosal barrier disruption—central features in the pathogenesis of HEM.

Pathway enrichment analysis using Reactome further supports the biological significance of *ANO1*. The rs2186797 variant was significantly associated with three pathways: Stimuli-sensing channels, Ion channel transport, and Transport of small molecules.[10,15,18,19] These pathways are integral to epithelial secretion, membrane excitability, and vascular tone regulation, supporting the mechanistic relevance of *ANO1* in HEM development. The Stimuli-sensing channels pathway, in particular, implicates *ANO1* in transducing extracellular cues into smooth muscle responses, aligning with clinical observations of altered rectal sensation and motility in HEM patients

In contrast, the *SRPX* gene, while showing possibly damaging PolyPhen-2 predictions for rs35318931 (score range: 0.568–0.83), did not demonstrate consistent deleterious predictions by SIFT and lacked significant enrichment in relevant biological pathways. *SRPX* encodes a Sushi repeat-containing extracellular matrix protein and has been linked to ECM remodeling and inflammation in other disease contexts.[23,24] Its moderate expression in fibroblasts and visceral adipose tissue, as reported in GTEx[14], may suggest a role in connective tissue remodeling—a factor known to contribute to hemorrhoid development. However, the absence of enrichment in pathways directly linked to HEM pathophysiology limits the strength of this association.

The *ABO* gene, associated with rs8176746, exhibited benign predictions across both SIFT and PolyPhen-2 analyses. Although the missense variant appears functionally neutral, *ABO* blood group polymorphisms have been associated with cardiovascular disease, thrombotic disorders, and gastrointestinal bleeding.[1,25-27] These epidemiological links suggest that the gene's involvement in HEM may stem from systemic vascular effects rather than direct protein function.

Taken together, these findings highlight *ANO1* as the most functionally and biologically relevant gene in HEM pathogenesis. It was the only gene to show concordant deleterious predictions across both in silico tools, substantial expression in smooth muscle-rich tissues, and significant pathway enrichment in Reactome. These characteristics underscore *ANO1*'s role in ion transport and smooth muscle physiology, processes central to the development of HEM. In contrast, while *SRPX* and *ABO* may have secondary or indirect roles, they lack the consistent integrative evidence observed with *ANO1*.

Despite these promising insights, this study has several limitations. The in silico predictions generated by SIFT and PolyPhen-2, while informative, require experimental validation to confirm their functional effects. Moreover, allele frequency data from the 1000 Genomes Project may not fully capture genetic variation in underrepresented populations.[13] The absence of transcriptomic or proteomic validation from hemorrhoidal tissue samples also limits mechanistic interpretation. Future studies should incorporate functional assays and diverse population cohorts to validate these findings and clarify the genetic basis of HEM, consistent with recent genomic network analyses that have begun to identify repositionable and druggable targets for hemorrhoid treatment.[28] Nevertheless, this study serves as a hypothesis-generating effort that integrates genomic, structural, and pathway-level data to identify molecular targets potentially implicated in HEM pathogenesis. This integrative framework provides a systematic foundation for subsequent experimental validation and translational exploration of candidate genes such as *ANO1*.

Conclusions

The integrative analysis of HEM-associated missense variants using SIFT and PolyPhen-2 revealed that the SNP rs2186797 in the *ANO1* gene may have a significant functional impact, with SIFT consistently predicting a deleterious effect and PolyPhen-2 suggesting possible damage depending on the amino acid position. In contrast, variants in *ABO* (rs8176746) and *SRPX* (rs35318931) were predominantly predicted to be benign or tolerated, though PolyPhen-2 indicated possible damaging effects for *SRPX*. Pathway enrichment analysis further supported the functional relevance of *ANO1*, linking rs2186797 to biologically significant pathways such as Stimuli-sensing channels and Ion channel transport within the Transport of small mol-

ecules category. Collectively, these findings suggest that *ANO1* may play a key role in the molecular mechanisms of HEM development through its involvement in ion transport regulation.

Ethical approval

Not applicable

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalized human and animal cell lines were used in the present study.

Conflict of interest

The authors have declared that no competing interests exist.

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There has been no financial support for this work, which could have influenced its outcomes.

Use of AI

No use of AI was reported.

Data availability

All data used are referenced or included in the article.

Author contributions

Conceptualization: WA and LMI; design: WA; data collection and/or processing: WA and AS; analysis and/or interpretation: WA, AS, and LMI; literature search: WA, AS, LP, LMI, DD, II, and MYH; writing: AS; critical reviews: WA, AS, LP, LMI, DD, II, and MYH.

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