Genomic Risk Factors for Urethral Stricture: A Systematic Review and Gene Network Analysis

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OBJECTIVE To identify genes that may play a role in urethral stricture and summarize the results of studies that have documented variations in gene expression among individuals with urethral stricture compared to healthy individuals.

METHODS A systematic search was conducted in Cochrane, Ovid, Web of Science, and PubMed, limiting the results to articles published between January 1, 2000 and January 30, 2023. Only studies comparing the difference in gene expression between individuals with urethral stricture and healthy individuals utilizing molecular techniques to measure gene expression in blood, urine, or tissue samples were included in this systematic review. Gene network and pathway analyses were performed using Cytoscape software, with input data obtained from our systematic review of differentially expressed genes in urethral stricture.

RESULTS Four studies met our criteria for inclusion. The studies used molecular biology methods to quantify gene expression data from specimens. The analysis revealed gene expressions of *CXCR3* and *NOS2* were downregulated in urethral tissue samples, while *TGFB1*, *UPK3A*, and *CTGF* were upregulated in plasma, urine and urethral tissue samples, respectively, in patients with urethral stricture compared to healthy controls. The analysis demonstrated that the most significant pathways were associated with phosphoinositide 3-kinase (PI3 kinase) and transforming growth factor beta 1/suppressor of mothers against decapentaplegic (TGF-β1/SMAD) signaling pathways.

CONCLUSION This systematic review identified gene expression variations in several candidate genes and identified underlying biological pathways associated with urethral stricture. These findings could inform further research and potentially shift treatment and prevention strategies for urethral stricture. UROLOGY 184: 251–258, 2024. © 2023 Elsevier Inc. All rights reserved.

Urethral stricture is caused by scarring that narrows the urethral lumen, resulting in symptoms such as weak stream, painful urination, and poor quality of life.[1,2] The incidence of urethral strictures in males over the age of 55 is approximately 1%, resulting in over 5000 inpatient visits annually.[3-5] The incidence of urethral stricture in men increases significantly above this age,[6] and women are rarely afflicted with the condition.[5] Of the women who present with voiding complaints, it is estimated that urethral stricture effects only 0.1%-1%.[5] Current treatment options include a choice between (1) minimally invasive (endoscopic) intervention historically associated with high recurrence rates or (2) highly effective but lengthy, complex open reconstructive surgery.[7-9] Repeated endoscopic intervention can exacerbate the stricture, potentially turning a curable condition into a chronic disease. Graft-based urethral reconstruction exhibits excellent long-term outcomes, but are limited because the procedure requires highly experienced reconstructive urologists and complications include donor tissue limitations and donor site morbidity.[10,11]

One of the most widely accepted explanations for the underlying pathophysiology of urethral stricture is the abnormal accumulation of fibrous tissue. This pathological state, spongiofibrosis, is typically characterized by a notable increase in collagen-rich connective tissue and fibroblasts, which appear to replace the corpus spongiosum.

Funding Support: *This research received no external funding or financial support.*

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Submitted: August 15, 2023, accepted (with revisions): December 14, 2023

This compressible erectile tissue encapsulates the urethra, and the emergence of these alterations is generally construed as a reflection of the body's innate response to injury. This complex process involves the accumulation of collagen and other extracellular matrix (ECM) components. This process commences with an inflammatory response and culminates with scar tissue formation.[12,13]

There is a possible genetic predisposition for urethral stricture,[14] although further comprehensive investigation is needed. Uncovering genes associated with urethral stricture could pave the way for novel diagnostic markers and innovative molecular treatments. Comparative gene expression analysis has emerged as a valuable research tool to systematically explore the molecular mechanisms implicated in disease initiation, progression, and prediction.[15] For example, a multigene urine test was developed utilizing gene expression analysis for the detection and stratification of bladder cancer in patients presenting with hematuria.[16] This review summarizes the findings from studies that examined gene expression levels in human tissues linked to urethral stricture. Additionally, it identifies potential gene correlations and networks, shedding light on the functional pathways involved.

METHODOLOGY

Search Strategy

We performed a comprehensive literature review to select articles investigating the connection between genetics and urethral stricture. This study was preregistered on the Open Science Framework (OSF) (https://osf.io/8b4n2). The search process adhered to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines and established protocols for conducting systematic reviews.[17] Our institution's academic librarian (Amber Stout) developed a systematic search plan to identify publications exploring genetics and urethral stricture. The search utilized multiple databases such as Cochrane, Ovid, Web of Science, and PubMed and was confined to manuscripts between January 1, 2000, and January 30, 2023. Studies published in languages other than the English language were excluded from screening. The search was completed by means of medical subject headings (MeSH) terms such as "urethral stricture," "genetics," "genetic variation," "genetic predisposition," and others. The specific search methods used can be found in Supplementary Table 1. Two authors (I.I. and T.R.W.) examined the resulting library using Covidence to identify relevant studies for this review. Covidence is a systematic review management tool to streamline the literature review process.[18]

Study Selection

Our analysis focused on gene expression data from patients with urethral stricture (Fig. 1). We included case-control studies to compare the difference in gene expression between patients with urethral stricture and healthy individuals. Studies that measured gene expression using molecular techniques on blood, urine, and tissue samples were included. Additionally, studies that focused on gene polymorphism were excluded as our focus was on understanding the distinct gene expression patterns in individuals with urethral stricture compared to healthy individuals.

Evaluation of the Studies for Potential for Bias

We utilized two methods to determine study quality to evaluate the articles effectively. First, we employed the Ottawa-Newcastle Scale (ONS).[19] This scale is a widely used tool for determining the quality of studies and ensuring that only studies of a high standard are considered. In addition to the ONS, the Joanna Briggs Institute (JBI) critical appraisal tool[20] was also used to evaluate the quality of the included manuscripts. Our evaluation of the included studies indicates that while cases and controls were appropriately matched, criteria for identification were consistently applied. There were inconsistencies in identifying confounding factors across the studies. Despite this, the outcomes were assessed reliably, and proper statistical analyses were used.

Data Collection

After the selection process, we utilized a standardized form to condense the key aspects of each study. Criteria included details such as the reference, study design, sample type, results summary, and analytical methods employed. The relevance of each gene to urethral stricture was determined based on the consistency of its expression through analytical and statistical techniques across the studies.

Analysis of Gene Networks

The Database for Annotation Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) is an online tool designed to facilitate exploratory visualization and discovery through functional classification and biochemical pathway mapping.[21] In our study, we utilized DAVID to classify gene ontology (GO), which provides functional descriptions of specific genes. By associating genes with corresponding GO terms, we leveraged DAVID to distribute GO annotations and enhance our understanding of gene functions.

GeneMANIA (https://genemania.org/) is a database and web-based tool that allows researchers to analyze and understand the relationships between genes in a biological system.[22] The constructed network emphasizes specific genes and assigns scores based on their interconnections in relation to the genes initially listed. In addition to the gene network analysis, genes were uploaded to the Cytoscape (https://cytoscape.org/) application to examine relationships and connections between proteins in a group of signaling network databases and interaction networks.[23] The network was further expanded by incorporating additional genes with

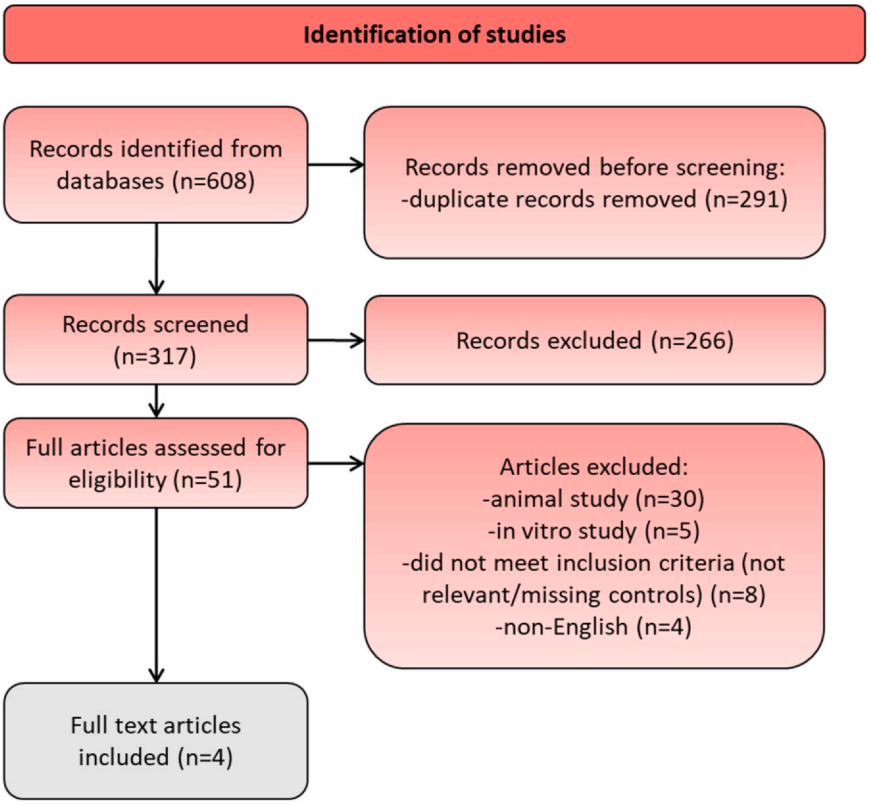

Figure 1. Flowchart of study selection and screening process. (Color version available online.)

the most significant number of interactions and the highest confidence level.

RESULTS

Systematic Review Results

The literature search produced 51 articles (Fig. 1). Four publications met the criteria for inclusion. The quality of the included studies was evaluated as fair to good based on assessment using the JBI critical appraisal checklist (Supplementary Table 2) and ONS scale (Supplementary Table 3) criteria in the systematic review. Gene expression was evaluated by using polymerase chain reaction (n = 2),[24,25] immunohistochemistry (n = 2), [25,26] flow cytometry (n = 1),[24] enzyme-linked immunosorbent assay (ELISA) (n = 2),[24,27] and Western blot (n = 1)[25] (Table 1). Our analysis revealed that the gene expressions of C-X-C motif chemokine receptor 3 (*CXCR3*)[24] and nitric oxide synthase 2 (*NOS2*)[26] were reduced, while transforming growth factor beta 1 (*TGFB1*),[24] uroplakin 3A (*UPK3A*),[27] and connective tissue growth factor (*CTGF*) were increased in patients with urethral stricture compared to healthy controls (Supplementary Table 4).

Gene Network Analysis

To investigate gene network connections, we employed GeneMANIA. The associations between the genes are depicted in a network diagram, with the lines in different colors representing the nature of the connection (Supplementary Fig. 1). Significant interactions were noted between the genes we identified as differentially expressed, such as physical interactions and coexpression.

Functional Analysis

Functional analysis showed that most of the genes have similar annotations related to the arginine metabolic process, glutamine family amino acid catabolic process, reactive oxygen species, regulation of purine nucleotide biosynthetic process, regulation of cyclase activity, and oxidoreductase activity (Table 2 and Supplementary Table 4).

Pathway Analysis

The results of the WikiPathways analysis demonstrated that the most influential pathways were related to the phosphatidylinositol 3-kinase (PI3K) and transforming growth factor beta/suppressor of mothers against decapentaplegic (TGF-β1/SMAD) signaling pathways.

Analysis of the Network of Interactions Between Proteins

The network analysis revealed a significant upregulation in connections between the genes transforming growth factor beta 3 (*TGFB3*), vimentin (*VIM*), endoglin (*ENG*), and bone morphogenetic protein 4 (*BMP4*) (Supplementary Fig. 2). This was indicated by a PPI enrichment *P*-value of 1.23e-2 and a similarity score of

Table 1. Key findings of the studies included in the systematic review.

Reference	Study Design	Sample Type	Results Summary	Analytical Methods Used
Cavalcanti et al[26]	Case control	Strictured human bulbar urethras (n = 33) Controls (n = 8)	Compared to normal urethral tissue, the strictured bulbar urethra showed a decrease in iNOS immunoreactivity. The degree of spongiofibrosis correlated with the decrease in iNOS immunoreactivity.	Immunohistochemistry
Zhang et al[25]	Case control	Strictured scar tissue (n = 12) Normal urethral tissue (n = 6)	The number of *CTGF* mRNA copies in tissue from patients with urethral stricture was significantly upregulated.	Real-time PCR Immunohistochemistry Western blotting
Szymanska et al[27]	Case control	Plasma samples from urethral stricture (n = 44) Controls (n = 32)	In the urethral stricture group, only the plasma *UPKIIIa* concentration differed significantly from the control.	ELISA
Xie et al[24]	Case control	Urine samples from patients with urethral stricture (n = 50) Recurring stricture treated by cystostomy (n = 50) Age and gender-matched healthy people (n = 50)	Urethral stricture patients had significantly elevated levels of *TGFB1* and decreased expression of *CXCR3*. The study revealed a crosstalk between *TGFB1* and *CXCR3* signaling in the regulation of urethral fibrosis.	ELISA Flow cytometry RT-PCR

iNOS, inducible nitric oxide synthase.

0.08, there is a statistically significant indication of biological connectivity among these proteins. The connections between the four genes studied are related to the positive regulation of the collagen biosynthesis process, arginine catabolic process, angiogenesis, response to decreased oxygen levels, wound healing involved in an inflammatory response, TGF-β1/SMAD protein complex assembly, positive regulation of fibroblast migration and negative regulation of macrophage cytokine production.

DISCUSSION

Our systematic review analysis showed changes in the gene expression of several key genetic immune response regulators in urine, plasma, and urethral tissue samples from patients with urethral stricture. The gene expressions of *CXCR3* and *NOS2*[26] were found to be downregulated, while *TGFB1*, *UPK3A*, and *CTGF*[25] were upregulated (Fig. 2). These gene expression changes are associated with several of the most influential pathways, such as the PI3 kinase and TGF-β1/SMAD signaling pathway.

Downregulated Genes

C-X-C Motif Chemokine Receptor 3 (CXCR3). The *CXCR3* gene codes for a protein that acts as a receptor on the surface of cells and is known to play a role in tumor migration.[28] The *CXCR3* gene has varying effects on the efficiency of chemotaxis in inflammation by triggering the activation of interferon-gamma (*IFN-γ*).[29] The activation of *CXCR3* can induce apoptosis in the vascular endothelium, leading to the inhibition of angiogenesis.[29] A study reports that chronic hypoxia increases the expression of the *CXCR3* gene and is implicated as a crucial component in the physiological transition involved in mitigating and possibly reversing early-stage fibrosis.[30] It was observed that the activity of the *CXCR3* gene was reduced in urine samples collected from patients suffering from urethral stricture.[24]

Nitric Oxide Synthase 2 (NOS2)

The *NOS2* gene codes for an enzyme that generates nitric oxide, an essential molecule involved in various cell functions such as metabolism and hypoxia response.[31] Suppressing *NOS2* decreases inflammation during the early stages, enhancing inflammation and fibrosis in the long run.[32] A notable reduction in *NOS2* gene expression in the strictured bulbar urethra suggests that the human urothelium can produce significant amounts of *NOS2*.[26] This enzyme seems to be the primary mediator for urethral dilation independent of adrenergic and cholinergic mechanisms. A decreased expression of *NOS2* could be a fundamental underlying mechanism in urethral stricture, which impedes the relaxation of smooth muscle in the urethra. Narrowing of the urinary tract caused by stricture disease leads to reduced flow and heightened pressure in the urinary system. Thus, nearby tissues may increase the expression of genes that induce the relaxation of smooth muscles. This could be an effort

Table 2. The top 3 clusters are accompanied by representative enriched terms, determined through genetic analysis.

GO	Category	Description	Count	%	Log10 (P)	Log10 (q)
GO:0036293	GO Biological Processes	Response to decreased oxygen levels	3	60.00	− 5.04	− 1.00
GO:0001525	GO Biological Processes	Angiogenesis	3	60.00	− 4.87	− 1.00
GO:0006954	GO Biological Processes	Inflammatory response	3	60.00	− 4.21	− 0.71

The "Count" indicates the number of genes from the user-provided lists that are associated with the genetic analysis method. The "%" represents the percentage of all user-provided genes that are linked to a specific ontology term (only genes with at least one ontology term annotation are considered). "Log10(P)" denotes the logarithm in base 10 of the *P-value, while "Log10(q)" represents the logarithm in base 10 of the adjusted P-value after multiple testing.*

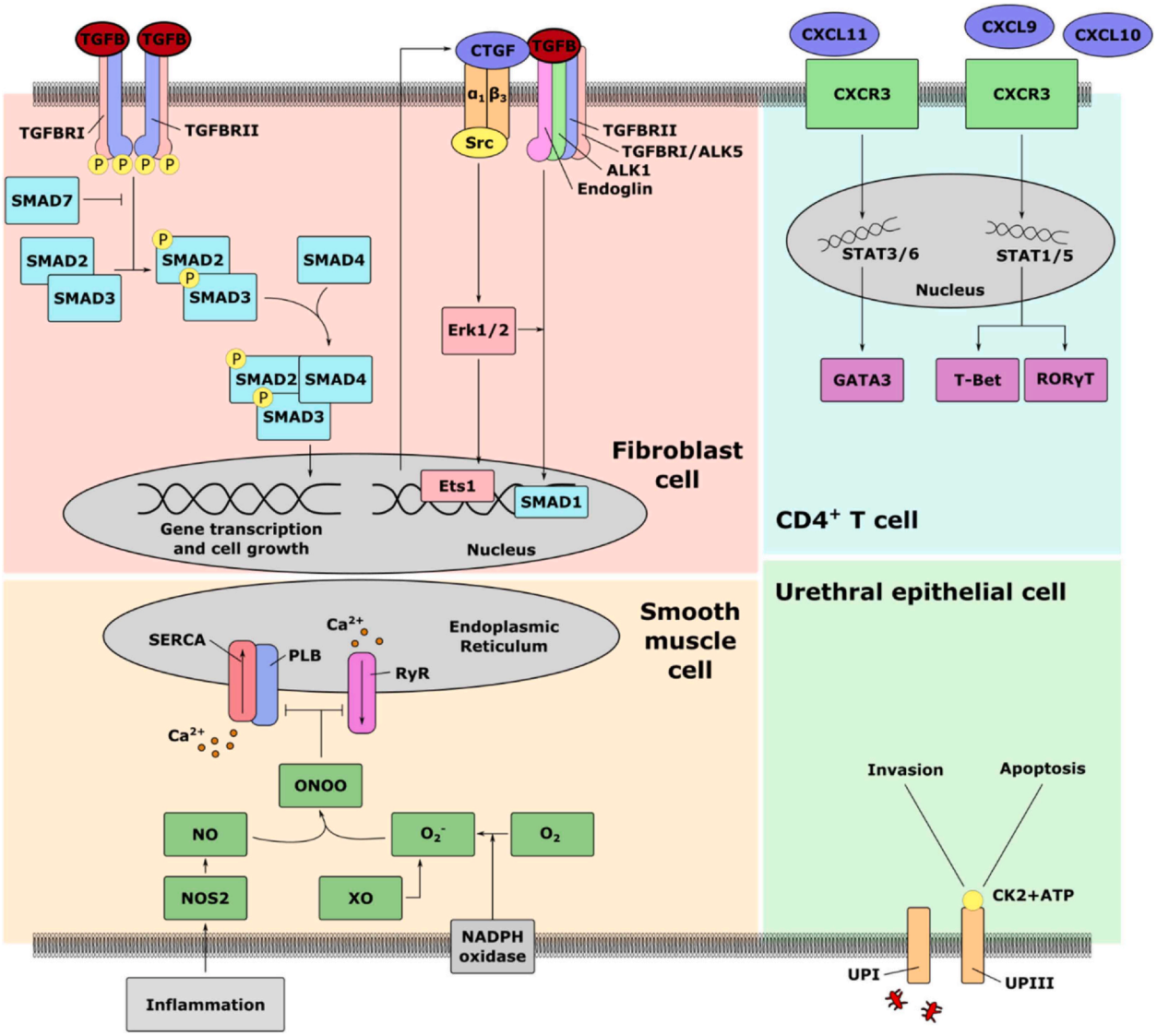

Figure 2. Overall summary of significant genes (*CXCR3, NOS2, TGFB1, UPK3A, CTGF,* and *NOS2*) and their associated connections. (Color version available online.)

to alleviate the blockage and improve the obstruction in the urinary tract.

Upregulated Genes

Transforming Growth Factor Beta 1 (TGFB1). *TGFB1* is pivotal in biological functions including immunoregulation, angiogenesis, facilitating wound repair, and contributing to the development of the urinary system.[33] It holds significant sway over the process of mesenchymal differentiation and is integral in the creation of the ECM.[34,35] *TGFB1* is a crucial agent in inducing urethral fibrosis.[36] In hypoxia, the TGF-β1/SMAD signaling pathway is stimulated through hypoxia-

inducible factor 1-alpha (*HIF-1α*), further encouraging collagen deposition.[37] Patients with urethral stricture exhibit enhanced expression of the *TGFB1* gene.[24] A more in-depth investigation is warranted to elucidate the role of heightened *TGFB1* gene expression in urethral stricture, likely requiring the development of an experimental model to help clarify its disease-causing role.

Uroplakin 3A (UPK3A)

The protein known as the *UPK3A* gene produces Uroplakin-3a and is predominantly located in the inner membrane of the urinary bladder.[38] Here, it enhances the membrane's resilience and expandability. Additionally, it can be found in the renal pelvis, ureter, and prostatic urethra. The *UPK3A* gene contributes to the development of kidneys, differentiation of epithelial cells, regulation of sodium and potassium ions, and the shaping of cells.[39] In patients with urethral stricture, *UPK3A* has been observed to be upregulated in plasma samples.[27] This series of findings imply a potential involvement of *UPK3A* in the inflammatory response associated with urethral stricture.

Connective Tissue Growth Factor (CTGF)

The cellular communication network factor 2 (*CCN2*) gene is situated on the 6th chromosome at 6q23.2 and is also known as *CTGF*.[40] It stimulates myofibroblasts and promotes their production and restructuring of ECM proteins.[41] The *CTGF* gene's role in these diseases may be due to its ability to amplify the fibrotic impacts of *TGF-β*. *CTGF* gene has been observed to be overexpressed in patients suffering from urethral stricture disease. The quantity of *CTGF* messenger ribonucleic acid (mRNA) in tissue samples from patients with urethral stricture was significantly increased.[25] The results from the network analysis provide insights into potential genetic underpinnings of urethral stricture. The study highlights a notable upregulation in interactions among four genes: *TGFB3*, *VIM*, *ENG*, and *BMP4*. TGFB3 is involved in regulating cytokine-stimulating fibrosis and modulating cell adhesion.[42] On the other hand, VIM is critical in sustaining cellular structure.[43] ENG is a component of the TGF-beta receptor complex,[44] while BMP4 appears crucial in the activation of autophagy and subsequent inhibition of apoptosis.[45] The observed increase in their interactions suggests that complex biological processes may lead to the formation of urethral stricture. Significant findings include the gene connections related to wound healing in the context of an inflammatory response, the assembly of the TGF-β1/SMAD protein complex, positive regulation of fibroblast migration, and negative regulation of macrophage cytokine production. The observed genetic interconnections present a complex interplay of biological processes that possibly contribute to the pathophysiology of urethral stricture.

Our findings suggest that the PI3K and TGF-β1/SMAD signaling pathways are relevant to urethral strictures. The PI3K pathway, crucial for inflammation and cell survival, may enhance fibrosis, a key feature of urethral stricture, by promoting immune responses and influencing wound healing and tissue remodeling.[46,47] TGF-β can also stimulate the production of matrix metalloproteinases, potentially leading to abnormal tissue remodeling.[48] Notably, the PI3K and TGF-β1/SMAD pathways may play a role in urethral stricture development by interacting and enhancing fibrotic effects, thus amplifying the overall fibrotic response. This interaction contributes to the proliferation of resident fibroblasts and collagen deposition, both processes sustained by the influence of TGF-β and dependent on the subsequent activation of the PI3K signaling pathway. These findings underline the complex and interconnected nature of the molecular mechanisms driving excessive scar formation and suggest that further research is necessary to understand the pathways associated with urethral stricture.

Previous studies identified associations between chromosome 19 anomalies and various urological disorders, including hydronephrosis, hydroureter, and kidney malformations.[49,50] However, our systematic review did not find a direct link between anomalies on chromosome 19 and urethral stricture. This could be an avenue for further genetic investigations into the development of urethral stricture.

Translational migration, a process integral to cellular adaptation and protein synthesis,[51] may have implications for tissue remodeling and fibrosis characteristic of stricture formation. While our initial review focused on direct genetic correlations, we acknowledge that gene expression and subsequent protein localization are also crucial to understanding the complex pathophysiology of urethral stricture. Future studies could explore how alterations in translational migration, perhaps influenced by genetic risk factors, contribute to the aberrant cellular processes leading to this condition.

This study has several limitations that should be acknowledged. One such limitation is the inclusion of a limited number of studies with heterogeneity. Additionally, the exclusion of studies primarily focused on gene polymorphism narrowed the scope of our research to understanding distinct gene expression patterns in individuals with urethral stricture compared to healthy individuals. Another limitation pertains to the small sample size in some of the included studies and the absence of information regarding the severity of urethral stricture in the patients included. Many of the available studies and datasets surveyed also lacked important details, such as the precise location from where the tissues were collected and whether the cases examined were chronic or acute. These missing pieces of information are particularly relevant as the duration of the stricture can influence the up-and-downregulation of genes, which may include nonspecific alterations related to inflammatory responses. In future investigations, it is recommended to thoroughly incorporate these factors, as they are critical for a complete analysis evaluating the relationship between tissue, urine, and blood gene expression changes and the development of urethral stricture.

It should be noted that any differences in gene expression levels observed in patients with urethral stricture compared to healthy individuals may not necessarily play a causal role in urethral stricture development. Instead, they may be a consequence of the condition itself. Therefore, utilizing these differences as potential biomarkers or predictive tools presents a challenge, as it would require patients to provide samples of their urethral tissue. However, identifying these genes in our study could prove valuable in the future for developing additional studies investigating new therapies or monitoring treatment responses. Despite these limitations, our systematic review is the first to highlight genes that exhibit differential expression in urethral stricture when compared to healthy controls, thus laying the foundation for a new study on this topic. It underscores several related genetic alterations and pathways as potential targets based on the limited current literature. Additionally, genomic research has significant diagnostic and therapeutic implications, as it can help in predicting disease outcomes and serve as a biomarker for various conditions.[52] Specifically for urological applications, genomic research has resulted in accurate and reliable biomarker-based evaluation methods. With these tools, clinicians can gain valuable information without the need for more invasive procedures.[16] Hence, the four articles included in our study represent an early understanding of urethral stricture.

CONCLUSION

The development of urethral stricture appears connected to angiogenesis, the excessive formation of fibrous tissue, and inflammation. It is crucial to conduct further investigations on human genes associated with these factors. The findings of this systematic review have the potential to establish the basis for future targeted therapies and the discovery of new biomarkers for treatment, early detection, and potentially predicting and preventing the development of urethral stricture in clinical practice.

Declaration of Competing Interest

None.

Acknowledgment. The authors express their sincere gratitude to Amber Stout, an expert librarian at University Hospitals Cleveland Medical Center, for her invaluable contribution in conducting the systematic review search for this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.urology.2023.12.014.

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