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A systematic review and in silico study of potential genetic markers implicated in cases of overactive bladder

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Abstract

OBJECTIVE: The contribution of genetic factors to the presence of an overactive bladder is recognized. This study aimed to (1) assemble and synthesize available data from studies assessing differential gene expression in patients with overactive bladder vs controls without overactive bladder and (2) determine possible correlations and functional pathways between genes.

DATA SOURCES: We searched PubMed, Ovid or Medline, and Wiley Cochrane Central Register of Controlled Trials databases between January 1, 2000, and December 15, 2021.

STUDY ELIGIBILITY CRITERIA: Studies were included if gene expression was detected and quantified using molecular approaches performed on human bladder tissue specimens directly and excluded if the gene expression analysis was carried out from blood and urine specimens alone.

METHODS: A systematic review was completed to identify publications that reported differently expressed gene candidates among patients with overactive bladder vs healthy individuals. Gene networking connections and pathway analysis were performed employing Metascape software, where inputs were identified from our systematic review of differentially expressed genes in overactive bladder.

RESULTS: A total of 9 studies were included in the final analysis and 11 genes were identified as being up-regulated (purinergic receptor P2X 2 [P2RX2], smoothelin [SMTN], growth-associated protein 43 [GAP43], transient receptor potential cation channel subfamily M member 8 [TRPM8], cadherin 11 [CDH1], gap junction protein gamma 1 [GJC1], cholinergic receptor muscarinic 2 [CHRM2], cholinergic receptor muscarinic 3 [CHRM3], and transient receptor potential cation channel subfamily V member 4 [TRPV4]) or down-regulated (purinergic receptor P2X 2 [P2RX3] and purinergic receptor P2X 5 [P2RX5]) in patients with overactive bladder. Gene network analysis showed that genes are involved in chemical synaptic transmission, smooth muscle contraction, blood circulation, and response to temperature stimulus. Network analysis demonstrated a significant genetic interaction between TRPV4, TRPM8, P2RX3, and PR2X2 genes.

CONCLUSION: Outcomes of this systematic review highlighted potential biomarkers for treatment efficacy and have laid the groundwork for developing future gene therapies for overactive bladder in clinical settings.

Keywords

gene expression; genomics; overactive bladder; systematic review; urge urinary incontinence

Introduction

Overactive bladder (OAB) is a storage symptom syndrome characterized by urgency, with or without incontinence, in the absence of urinary tract infection or another obvious pathology.[1] Recent reports indicate between 20% and 50% of women are affected by OAB in the United States alone.[2,3] Patients with OAB often suffer social, sexual, occupational, and psychological effects, which severely affect their quality of life.[4] The pathophysiology of OAB is multifaceted with various mechanisms. The influence of the cholinergic system, nitric oxide, and adrenergic mechanisms are crucial to regulating bladder filling and storage processes; moreover, up to 70% of OAB cases are considered idiopathic.[5] Although there exist several treatment options for OAB, success rates are highly variable, likely reflecting the multifaceted nature of OAB phenotypes.[6,7] Genetic factors are implicated in the development of OAB, but, to date, there has been limited evaluation of the genetic components underlying OAB.[8-10]

The urothelium directly communicates with suburothelial afferents acting as luminal sensors. Low pH, high potassium concentration in the urine, and increased osmolality can affect sensory nerves. Detrusor tissue from patients with idiopathic instability shows increased electrically evoked contractions but normal sensitivity to muscarinic agonists. The large conductance, voltage- and calcium-activated K+ channel, known as the big potassium (BK), is highly expressed on urinary bladder smooth muscle cells and regulates bladder detrusor muscle function, as has been demonstrated using gene transfer for the BK channel for the treatment of OAB. Gene expression profiling enables the evaluation of the gene expression patterns and can identify many potential drug targets for treatment and biomarkers for treatment efficacy.[11-13] Comparative genomic studies between normal bladder and OAB-afflicted tissues may provide a more direct way to evaluate the OAB pathophysiology. The bladder's unique suitability for instillation gene therapies makes the gene therapies superior because of the endurance of bladder architecture histologically indistinguishable from normal controls without any evidence of cystitis or systemic spread of the infection.[14] The identification of OAB-associated genes may lead to groundbreaking biomarkers and novel gene therapy approaches.

Objective

This review aimed to summarize current evidence from studies evaluating differential gene expression levels between normal and OAB bladder tissues to determine genes associated with OAB and identify possible correlations and networks between genes and functional pathways.

Methods

Eligibility criteria, information sources, and search strategy

A systematic literature review was conducted to identify publications investigating genetic contributions in the development of OAB and assess potential targets in a prognostic and/or clinical therapeutic context. The search was performed in compliance with the Preferred Reporting Items for Systematic Reviews guidelines for systematic reviews.[15] The study

was preregistered in the international Open Science Framework to host our protocol (https://osf.io/knfud) and study data in a publicly available database.

A systematic search strategy was developed by an academic librarian (A.C.S.) at our institution to identify publications that identified differently expressed gene candidates among patients with OAB. The search was performed using PubMed, Ovid or Medline, and Wiley Cochrane Central Register of Controlled Trials. The search results were limited to research published between January 1, 2000, and December 15, 2021, to include as many pertinent articles as possible. The search was performed using Medical Subject Headings terms, such as “overactive bladder,” “urge urinary incontinence,” “detrusor overactivity,” “genetic,” “gene,” and “gene expression.” The comprehensive search strategies are available (Supplemental Table 1). Of note, 2 researchers (I.I. and P.M.) screened the generated library using Rayyan to discover pertinent studies that contributed to this review. Rayyan was used to accelerate the literature review process by operating machine learning technologies. It relies on initial input from the users in terms ofkey words and phrases,[16] and users (I.I and P.M) confirmed whether to include or exclude the returned studies.

Study selection

The inclusion criteria included primary research data examining gene expression analysis of patients with OAB vs controls without OAB. Studies were included if gene expression was detected and quantified using molecular techniques performed on bladder tissue specimens directly and excluded if the gene expression analysis was carried out from urine or blood specimens alone. In vitro and animal gene expression studies were excluded to clarify differentially expressed genes in humans alone. No other limitation was placed initially on data collection. All manuscript titles were screened for relevance, and eligible manuscripts were subjected to a full-text analysis. Figure 1 illustrates the overall manuscript selection approach in a flow diagram.

Data extraction

Following selection, a standardized form was used, with parameters that included study characteristics, such as reference, study design, study population and age, gene selection process, and analytical method used. The primary outcomes of this study were to report examined significant genes and different analytical methods used across studies. Genes were selected and considered to be related to OAB if their expression was consistently corroborated through all statistical and analytical methods (reverse transcriptase-polymerase chain reaction, immunohistochemistry, etc.) used within the included study.

Gene network analysis

Database for Annotation, Visualization, and Integrated Discovery (DAVID),[17] a web-accessible program, was employed for functional annotation of most significant genes. In addition to primary outcomes, GeneMANIA,[18,19] a user-friendly, real-time, multiple association network integration algorithm, was employed to create gene set functional hypotheses. A list of the most significant differentially expressed genes was uploaded to the GeneMANIA server. The network of genes displaying co-expression, physical interactions, co-localization, shared protein domains, and predicted interactions was generated. The

created network emphasizes genes and scores them based on comparative networks in the initial list.

Pathway analysis

In addition, for each given gene list, pathway and functional enrichment analyses have been carried out with the Gene Ontology Biological Processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using Metascape 3.[20] Protein-protein interaction (PPI) enrichment analysis was also carried out with the STRING, one of the most comprehensive PPI data sources. Only physical interactions in STRING (physical score of >0.132) were used. The resultant network contains the subset of proteins that form physical interactions with at least 1 other member in the list.

Assessment of risk of bias

The included studies were evaluated using the Newcastle-Ottawa Quality Assessment Scale[21] and defined using the Agency for Healthcare Research and Quality (AHRQ) standards (Supplemental Table 2). In addition, the Joanna Briggs Institute (JBI) crucial appraisal tool[31] was used to assess the relevance and results of the included manuscripts (Supplemental Table 3).

Results

Systematic review results

Study selection.—The literature search returned 130 studies, of which, only 9 met the inclusion criteria by presenting gene expression data from bladder tissue specimens that were quantified with molecular biology methods. These 9 studies were identified from the final literature search and included in the subsequent genetic analysis.

Study characteristics.—For the 9 included studies, genes were assessed and categorized as being either over- or underexpressed in patients with OAB vs normal patients. Genes with notable over- or underexpression were examined further for genetic associations, interactions between their coded proteins, and related pathways.

Risk of bias of included studies.—Overall, the included studies had fair to good quality as assessed by AHRQ standards (Supplemental Table 2). Included studies in the systematic review met the criteria of JBI critical appraisal checklist (Supplemental Table 3).

Synthesis of results between the studies.—A total of 9 genes were identified as overexpressed in OAB patients (purinergic receptor P2X 2 [P2RX2], smoothelin [SMTN], growth-associated protein 43 [GAP43], transient receptor potential cation channel subfamily M member 8 [TRPM8], cadherin 11 [CDH1], gap junction protein gamma 1 [GJC1], cholinergic receptor muscarinic 2 [CHRM2], cholinergic receptor muscarinic 3 [CHRM3], and transient receptor potential cation channel subfamily V member 4 [TRPV4]) and a total of 2 genes (purinergic receptor P2X [P2RX3] and purinergic receptor P2X 5 [P2RX5]) were reported with reduced expression. A summary of reported outcomes of the included studies can be found in Table 1.

Genetic analysis

Database for Annotation, Visualization, and Integrated Discovery functional analysis.—Functional analysis revealed that most up-regulated genes share common annotations or biology in the involvement of intrinsic components of the nuclear inner membrane, ligand-gated cation channel activity, nucleotide receptor activity, actin-mediated cell contraction, modulation of chemical synaptic transmission, calcium ion transmembrane transporter activity, complex of collagen trimers, cell-cell junction organization, acetylcholine receptor signaling, adenylate cyclase-inhibiting G protein–coupled receptor (GPCR) signaling, postsynaptic signal transduction, and G protein–coupled amine receptor activity. Moreover, the down-regulated genes share similar features in terms of regulation of cytosolic calcium ion concentration, protein autophosphorylation, inorganic cations import across the plasma membrane, extracellular ligand-gated ion channel activity, positive regulation of cytosolic calcium ion, and response to purine-containing compound (Table 2).

GeneMANIA network analysis.—We ran GeneMANIA to analyze significantly overexpressed (P2RX2, SMTN, GAP43, TRPM8, CDH11, GJC1, CHRM2, CHRM3, and TRPV4) and underexpressed (P2RX3 and P2RX5) genes to further predict network connections. Interactions between genes are displayed as a network diagram. The color of the line connecting the genes indicates the type of communication (yellow: shared protein domains [47.23%]; orange: predicted communication [17.10%]; red: physical interactions [16.64%]; purple: co-expression [12.86%]; blue: co-localization [6.06%]) (Figure 2).

Process enrichment analysis.—The most significant genes were found to be involved in chemical synaptic transmission, smooth muscle contraction, regulation of nervous system process, blood circulation, and response to temperature stimulus with the most statistically significant term within a cluster chosen to represent the cluster (Table 3).

Kyoto Encyclopedia of Genes and Genomes pathway analysis.—KEGG analyses reported significant pathways based on their log(*P*) values. The KEGG pathway analysis showed that the most significant genes were primarily involved in “chemical synaptic transmission,” “smooth muscle contraction,” “regulation of nervous system process,” “blood circulation,” and “response to temperature stimulus” pathways. Metascape displayed the top enrichment clusters for differentially expressed genes, which were discretely colored to encode *P* values of increasing statistical significance (Figure 3).

Protein-protein interaction network analysis.—The network showed significantly more interactions between the TRPM8, TRPV4, P2RX3, and P2RX2 genes with a PPI enrichment *P* value of 3.41×10^{-5}. Such an enrichment indicates the proteins, as a group, are at least partially biologically connected. The pathways between the constructed 4 genes were involved in “calcium signaling pathways” and “neuroactive ligand-receptor interaction” pathways (Figure 4). A schematic overview of up-regulated and down-regulated genes and their evidenced-based localization, functions, and connections can be found in Figure 5.

Comment

Principal findings

Up-regulated genes.

Purinergic receptor P2X 2.: The P2RX2 protein—coding gene located on chromosome 12 is of the purinoceptor family for adenosine triphosphate (ATP) and acts as a moderator for neuron to neuron and neuron to smooth muscle synaptic transmissions.[32] P2RX2 has been implicated in other processes, including sound perception, neuron action potential, regulation of ion concentrations, and ischemia.[33] P2RX2 overexpression has been associated with spinal injuries and hearing loss.[34] The appropriate presence of P2RX2 is necessary to maintain the proper functioning of the bladder and up-regulation of P2RX2 is crucial for the changes in purinergic innervation in idiopathic detrusor instability bladders.[22]

Smoothelin.: The SMTN gene, located on chromosome 22, is responsible for coding proteins expressed only in smooth muscle cells.[35] The SMTN gene is associated with the maintenance of the actin cytoskeleton and actin cytoskeletal dynamics.[36] SMTN is involved in smooth muscle cell contraction, and the analysis of smooth muscle cells shows SMTN levels are significantly increased in patients with OAB.[23]

Growth-associated protein 43.: Growth-associated protein 43 is membrane bound, localized to the internal surface of the growth cone membrane, and is highly expressed during axonal growth and regeneration.[37] GAP43 was up-regulated in degenerated primary afferent nerves during acute cystitis.[38] Overexpression of GAP43 was reported in patients with OAB, indicating that GAP43 might be involved in the regulation of neuronal degeneration and regeneration during inflammatory states.[25]

Transient receptor potential cation channel subfamily M member 8.: TRPM8 has a crucial role in influencing transmembrane calcium ion transport, ligand-gated calcium channels, and thermogenesis.[39] TRPM8 is typically activated in response to low temperature and is associated with dry eye disease[40] and obesity[41] in mouse studies. Overexpression of TRPM8 has been related to bladder activity, possibly through interactions with mechanosensitive C fibers.[42] A recent rat study suggested that combination therapy of TRPM8 antagonist and β3-adrenoceptor agonist or anticholinergic agent can be a potential treatment option for obtaining additive effects compared with monotherapy for OAB.[43] Marked increase of TRPM8-immunoreactive nerve fibers was detected in patients with OAB compared with controls without OAB.[26]

Cadherin 11.: Cadherins are cell surface glycoproteins that mediate Ca2+–dependent, hemophilic, cell-cell adhesion in epithelial tissues.[44] The CDH11 gene is responsible for encoding a cadherin superfamily type II classical cadherin.[45] Cadherin 11 up-regulation in suburothelial myofibroblasts in patients with overactive bladder may be significant in overactive bladder pathogenesis.[27]

Gap junction protein gamma 1.: Connexin proteins form gap junctions between adjacent cells to regulate cell-to-cell communications.[46] GJC1 gene, also known as Cx45, is

implicated in the initiation of inflammation through the activation of purinergic signaling pathways.[47] GJC1 relates to extracellular ATP signaling in the urothelium of patients with bladder overactivity and urge incontinence. Both Cx43 and Cx45 are expressed at low levels in normal detrusor. The up-regulation of Cx45 in the myofibroblast cell layer supports the idea that alterations in sensory signaling are also involved in the pathogenesis of idiopathic OAB.[28]

Cholinergic receptor muscarinic 2.: The CHRM2 gene fits into the superfamily of GPCRs. Muscarinic acetylcholine receptors (M1–M5) activate a multitude of signaling pathways essential for controlling neuronal excitability and feedback management of acetylcholine release.[48] Overexpression of CHRM2 was found in patients with persistent detrusor overactivity.[49] Increased expression of the CHRM2 gene in myofibroblastlike cells suggests a potential role in pathophysiological mechanisms and the therapeutic effect of antimuscarinic agents in OAB.[30-

Cholinergic receptor muscarinic 3.: CHRM3 gene is located on chromosome 1 and primarily responsible for smooth muscle contraction and glandular tissue secretion.[48] Similar to CHRM2, increased expression of the CHRM3 gene has potential pathophysiological mechanisms in idiopathic OAB.[30]

Transient receptor potential cation channel subfamily V member 4.: TRPV4 is mainly expressed in bladder basal and intermediate urothelial cells and functions as a Ca2+ influx pathway activated by hypotonic cell swelling.[50] TRPV4 mutation has also been identified in a spectrum of neuromuscular diseases that includes congenital distal spinal muscular atrophy, and hereditary motor and sensory neuropathy type IIC.[51] Up-regulation of this gene was reported to be related to the pathogenesis of OAB.[29]

Down-regulated genes.Purinergic receptor P2X 3.: P2RX3 is 1 of 7 genes encoding the purinoceptor gene family and is expressed by sensory and autonomic neurons. Underexpression of P2RX3 resulted in a reduction in pain sensation and inflammation, suggesting that it plays an essential signaling role in the nervous system.[52] Down-regulation of P2RX3 was associated with bladder hyporeflexia in 1 mouse study.[53] P2RX3 deficiency may impair the control of detrusor contractility and contribute to the pathophysiology of urge incontinence. Therefore, antagonists to P2RX3 may have therapeutic potential in the treatment of disorders of urine storage and voiding, such as OAB.[24]

Purinergic receptor P2X 5.: P2RX5 receptor gene, located on chromosome 17, is also a ligand-gated ion channel. It is located on chromosome 17 and plays a crucial role in skeletal muscle tissue regeneration.[54] The up-regulation of P2RX5 has been associated with T-cell activation, implying critical functionality in the immune system.[55] Down-regulation of the gene has been correlated to detrusor instability and impaired bladder control.[24]

This systematic review identified a total of 11 genes with potential involvement in the pathogenesis of OAB. GeneMANIA analysis highlighted, in particular, P2RX genes as having “predicted communication” between them, indicating possible links in up- and down-regulation of genes in this family. The network analysis revealed further interactions

between TRPV4, TRPM8, P2RX3, and P2RX2 genes. The pathways constructed between the 4 genes were involved in “calcium signaling pathways” and “neuroactive ligand-receptor interaction” (Figure 4). Opening voltage-dependent Ca2+ channels on the cell surface triggers bladder emptying contraction via a large influx of extracellular calcium. The activation of purinergic receptor genes was also implicated in bladder afferent hyperexcitability. Further exploration of this interaction network through molecular-level experimental studies may lead to understating the pathophysiology of the occurrence and development of OAB.

TRPV4 has been expressed mainly in bladder basal and intermediate urothelial cells and contributes to bladder filling detection. Reportedly, TRPV4 senses bladder urothelial cultured cell stretching, which is converted to ATP signals in the micturition reflex pathway during the storage phase. Up-regulation of TRPV4 might lead to a decreased sensation of bladder fullness via abnormal wall stretch-related signaling to afferent pathways and results in detrusor overactivity. An experimental study showed that intravesical activation of the cation channel TRPV4 improves bladder function in a rat model for detrusor underactivity.[56] Conversely, TRPV4−/− mice exhibit an abnormal voiding pattern and decreased urothelial stretch-evoked ATP release.[50] Currently, there is limited understanding of this difference between the 2 species. A study by Roberts et al[29] showed that urothelial tissue in human OAB bladders spontaneously releases more ATP than cohort non-OAB bladders. Up-regulation of TRPV4 caused a greater quantity of stretch-induced ATP release in the mucosa and detrusor smooth muscle from aging bladders of patients with OAB. In addition, aging pig bladder mucosa exhibited greater TRPV4-induced ATP release with age dependence.[29]

Our analysis revealed that TRMP8 was expressed in patients with OAB as part of a response to the temperature stimulus pathway. TRPM8 is a cold-activated ion channel that plays a crucial role in detecting environmental temperatures and is targeted by antagonists that may be useful for reducing cold hypersensitivity resulting from nerve damage.[57] It is noteworthy to mention that some people with OAB specifically report worsening symptoms with exposure to low temperatures, and this may represent a unique phenotype related to differential expression of TRMP8.[58]

In a previous animal model study, TRPM8 channel knockout mice had significantly less cold sensitivity.[59] In addition, TRPM8 in the dorsal root ganglion might play a role in urinary urgency induced by cold sensation.[60] Our study has highlighted further investigation of this gene as a new therapeutic opportunity for overactive bladder patients. In addition, this may lead to individualized treatments in patients with TRMP8 overexpression and could be a way to identify those patients with distinct phenotypes. Selective TRPM8 antagonists may provide valuable treatment for disorders related to the hyperactivity of bladder afferent nerves.

Comparison with existing literature

To date, most genomic studies[61,62] were used from urine and blood samples, not actual bladder tissue specimens. Of note, 1 study that included 37 reports highlighted the prevalence of nerve growth factors in urine and serum samples collected from patients.[61]

Furthermore, they noted the prevalence of signal transduction pathways and, to a lesser extent, inflammatory responses associated with urgency urinary incontinence (UUI). Interestingly, the C-reactive protein was noted to be present in the serum of patients with UUI, but it was not significantly present in urine samples. In terms of tissue markers, alterations in the expression of GAP43, P2X (1—7), and TRPV1 were found to be implicated in cases of UUI,[61] similar to the results of our findings.

Strength and limitations

The limitations of this study included the small sample size in some of the studies examined and the lack of information on the severity of OAB in the patients they analyzed. It should also be noted that we included studies where gene analysis was performed on bladder tissue specimens directly and excluded studies if the gene expression analysis was performed from urine and blood samples of patients vs healthy individuals.

Our initial aim was to run a meta-analysis based on publicly available Gene Expression Omnibus (GEO) data to categorize differentially expressed genes between controls without OAB and patients with OAB. GEO is a database repository of high-throughput gene expression data via hybridization arrays, chips, and microarrays. However, we could not find any available GEO datasets to perform a meta-analysis that could lead to robust findings for biomarker discovery studies. To the best of our knowledge, no optimized approach is established currently to run a meta-analysis using all molecular gene expression methods. Only microarray and RNA sequencing studies can be used to run meta-analysis using ImaGEO, ExAtlas, and Network Analyst web-based bioinformatics tools. Therefore, our gene expression analysis in the study was not an exhaustive representation of existing literature and might not be considered a deep analysis of available literature.

It is worth mentioning that any distinctions in gene expression level observed in patients with OAB vs controls without OAB are not necessarily involved in the development of OAB, which could be a consequence of the condition. Turning this into a potential biomarker or prediction tool might prove challenging, as it requires patients to give up bladder tissue. However, developing new treatments and tracking responses to treatment based on reported genes in this study could be a beneficial approach in the future. Despite these limitations, our systematic review highlighted differentially expressed genes in patients with OAB vs controls without OAB and emphasized several crucial genetic alterations as candidate targets from available reported literature.

Conclusions and implications

Further evaluation of the genes and pathways listed in our study would benefit researchers and clinicians. Our study did not conclude any clinical significance based on the in silico approach findings. Functional validation of potent trial targets and pathways needs to be further emphasized in vitro and using animal models. A better understanding of genes with poorer clinical outcomes might be prioritized for future targeting in clinical settings. Exploration of these specific pathways and gene interactions may be the key to unlocking the hidden mechanisms underlying the pathogenesis of OAB.

This systematic review identified differential expression of genes and gene-gene interactions among people with OAB, which may provide insight into distinct OAB phenotypes. Comprehensive experiments should be undertaken to evaluate the differential expression of these genes further. The outcomes of this systematic review may lay the groundwork for the development of future genes or other therapies and biomarkers for treatment efficacy of OAB in clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance

Why was this study conducted?

Although several studies have investigated genetic markers for overactive bladder (OAB) present in blood and urine, there is a distinct lack in the literature of tissue-level gene network analyses concerning OAB.

Key findings

Of note, 9 genes were found to be up-regulated (purinergic receptor P2X 2 [P2RX2], smoothelin [SMTN], growth-associated protein 43 [GAP43], transient receptor potential cation channel subfamily M member 8 [TRPM8], cadherin 11 [CDH1], gap junction protein gamma 1 [GJC1], cholinergic receptor muscarinic 2 [CHRM2], cholinergic receptor muscarinic 3 [CHRM3], and transient receptor potential cation channel subfamily V member 4 [TRPV4]) and 2 genes were down-regulated (purinergic receptor P2X 2 [P2RX3] and purinergic receptor P2X 5 [P2RX5]) in bladder tissues of patients with OAB.

What does this add to what is known?

This was a systematic review of genetic biomarkers associated with OAB at the tissue level. Study findings could offer a better means of assessing overactive bladder pathophysiology and provide direction for future analyses.

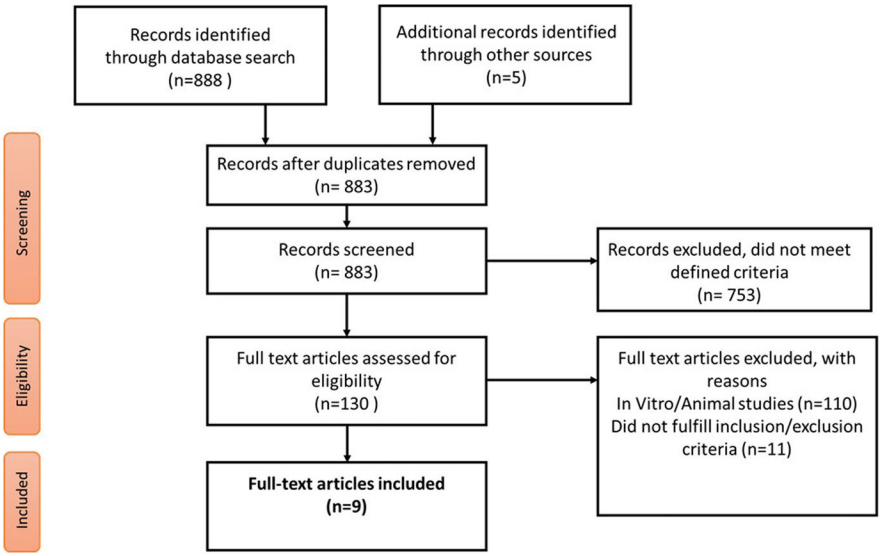

FIGURE 1. Flow diagram for study selection
Other sources indicate a Google search.

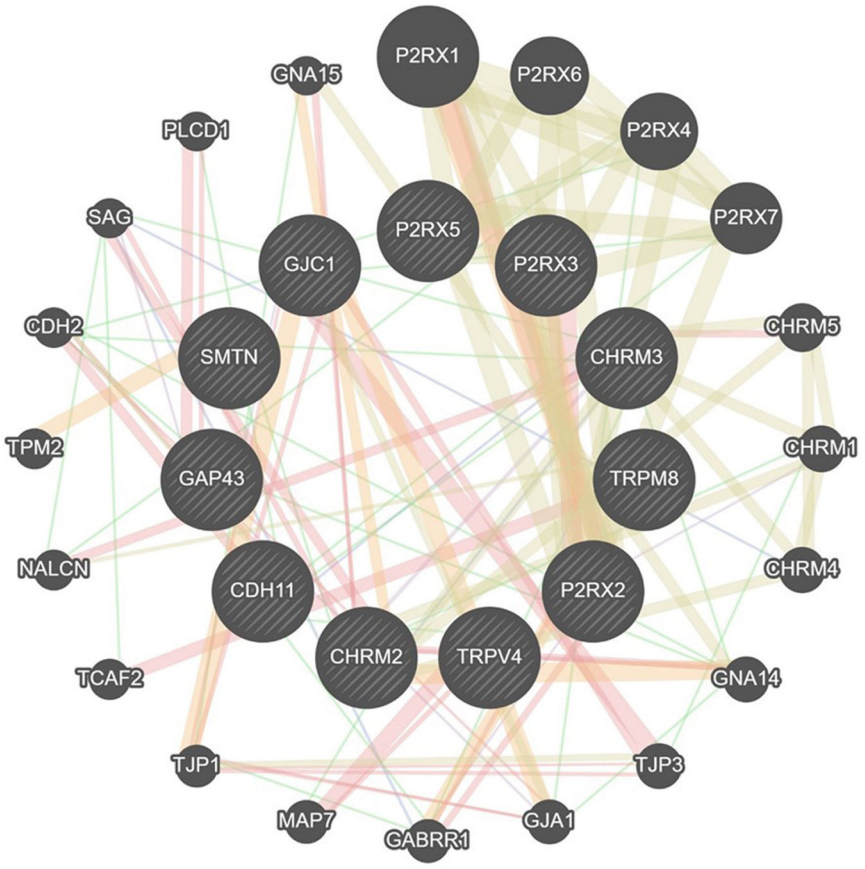

FIGURE 2. GeneMANIA interaction analysis for upregulated genes in OAB

The most significant genes (P2RX2, SMTN, P2RX3, P2RX5, GAP43, TRPM8, CDH11, GJC1, TRPV4, CHRM2, CHRM3) are depicted in *dash lines*. The color of the line connecting the genes indicates the type of communication (*purple*, co-expression; *red*, physical interactions; *Blue*, co-localization; *yellow*, shared protein domains; *orange*, predicted communication; *green*, genetic interactions).

CDH2, cadherin 2; *CDH11*, cadherin 11; *CHRM1*, cholinergic receptor muscarinic 1; *CHRM2*, chinergic receptor muscarinic 2; *CHRM3*, cholinergic receptor muscarinic 3; *CHRM4*, cholinergic receptor muscarinic 4; *CHRM5*, cholinergic receptor muscarinic 5; *GABRR1*, gamma-aminobutyric acid receptor subunit rho-1; *GAP43*, growth-associated protein 43; *GJA1*, gap junction protein alpha 1; *GJC1*, gap junction protein gamma 1; *GNA14*, guanine nucleotide-binding protein subunit alpha-14; *GNA15*, guanine nucleotide-binding protein subunit alpha-15; *NACLN*, sodium leak channel, nonselective; *MAP7*, microtubule associated protein 7; *P2RX*, purinergic receptor P2X; *PLCD1*, phospholipase C delta 1; *SAG*, S-antigen visual arrestin; *SMTN*, smoothelin; *TCAF2*, TRPM8 channel associated factor 2; *TJP1*, tight junction protein 1; *TJP3*, tight junction protein 3; *TPM2*, tropomyosin 2; *TRPM8*, transient receptor potential cation channel subfamily M member 8; *TRPV4*, transient receptor potential cation channel subfamily V member 4.

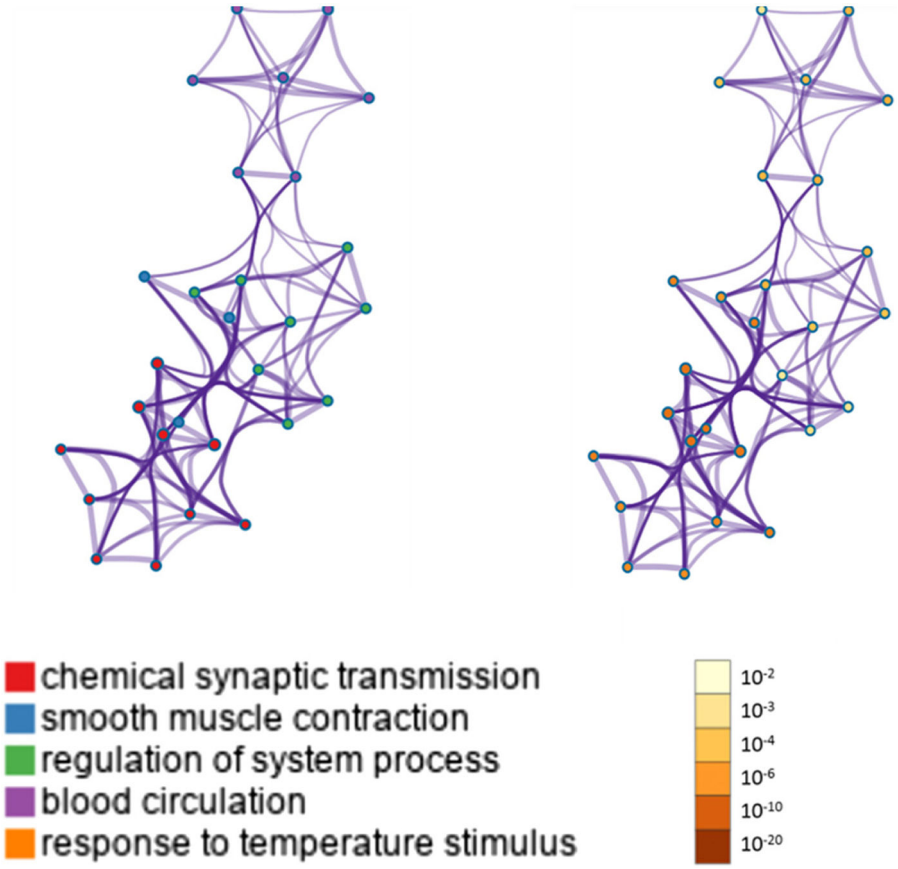

FIGURE 3. Metascape enrichment analysis for the included genes

Network of enriched terms colored by cluster ID, where nodes sharing the same cluster ID are typically close to each other (threshold: 0.3 kappa score; similarity score of > 0.3). ID, identification.

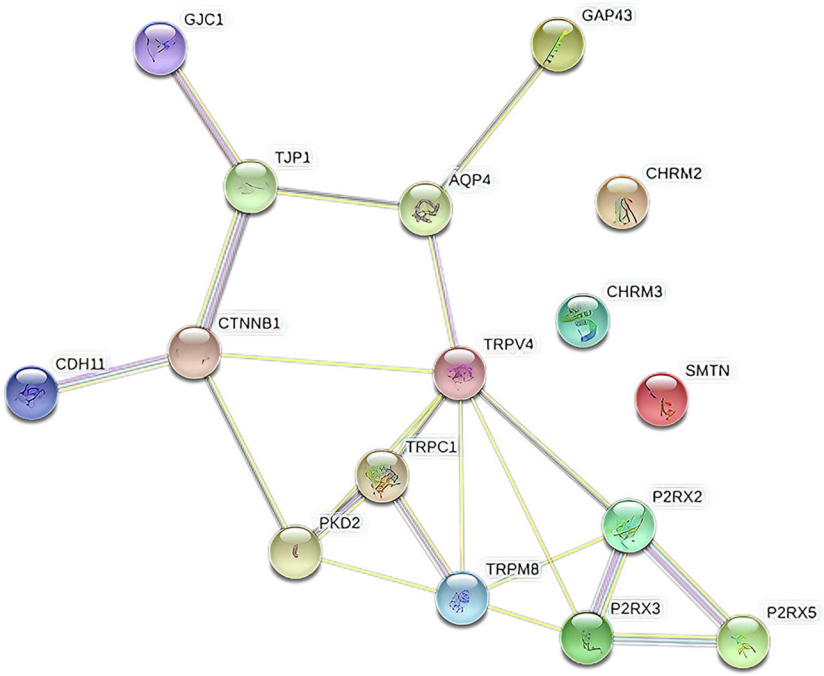

FIGURE 4. Protein-Protein Interaction Network based on studied genes

The network showed significantly more interactions between the TRPM8, TRPV4, P2RX3, and PR2X2 genes (number of nodes: 16; number of edges: 21).

AQP4, aquaporin-4; *CDH11*, cadherin 11; *CHRM2*, chinergic receptor muscarinic 2; *CHRM3*, cholinergic receptor muscarinic 3; *CTNNB1*, catenin beta 1; *GAP43*, growth-associated protein 43; *GJC1*, gap junction protein gamma 1; *P2RX*, purinergic receptor P2X; *PKD2*, polycystin 2, transient receptor potential cation channel; *SMTN*, smoothelin; *TJP1*, tight junction protein 1; *TRPC1*, transient receptor potential cation channel subfamily C member 1; *TRPM8*, transient receptor potential cation channel subfamily M member 8; *TRPV4*, transient receptor potential cation channel subfamily V member 4.

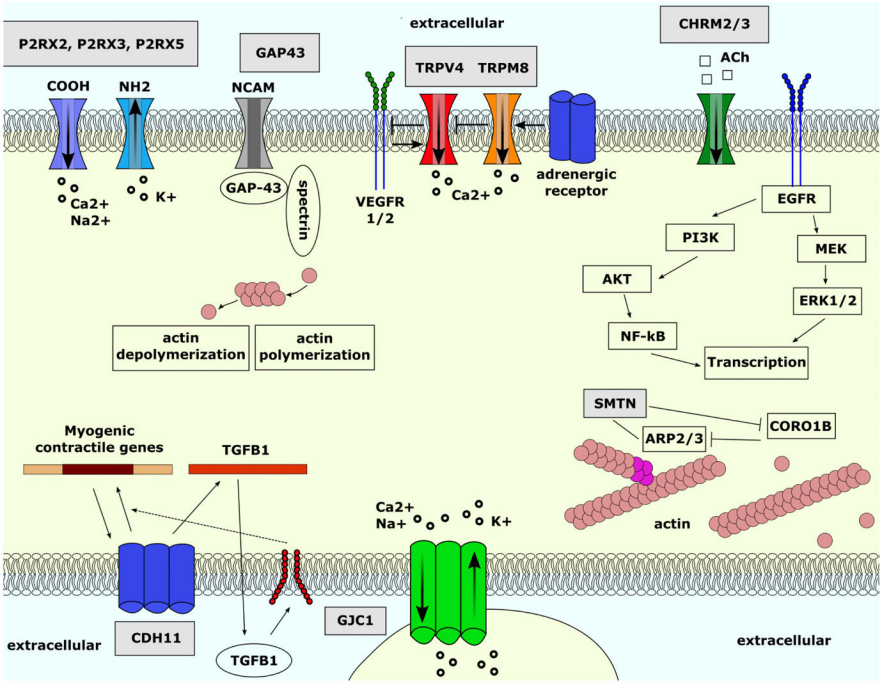

FIGURE 5. Overall summary of significant genes and their associated connections

Ach, acetylcholine *ARP2/3*, actin-related protein 2/3; *CDH11*, cadherin 11; *CHRM2*, chinergic receptor muscarinic 2; *CHRM3*, cholinergic receptor muscarinic 3; *CORO1B*, coronin 1B; *CTNNB1*, catenin beta 1; *EGFR*, epidermal growth factor receptor; *ERK1/2*, extracellular signal-regulated kinase 1/2; *GAP43*, growth-associated protein 43; *GJC1*, gap junction protein gamma 1; *NCAM*, beural cell adhesion molecule; *NF-κB*, nuclear factor kappa B; *P2RX*, purinergic receptor P2X; *PI3K*, phosphoinositide 3-kinases; *PKD2*, polycystin 2, transient receptor potential cation channel; *SMTN*, smoothelin; *TGBF1*, transforming growth factor beta 1; *TJP1*, tight junction protein 1; *TRPC1*, transient receptor potential cation channel subfamily C member 1; *TRPM8*, transient receptor potential cation channel subfamily M member 8; *TRPV4*, transient receptor potential cation channel subfamily V member 4; *VEGFR1*, vascular endothelial growth factor receptor 1; *VEGFR2*, vascular endothelial growth factor receptor 2.

TABLE 1

Summary of reported outcomes in the studies included

Reference	Study design	Study population and age (average/range)	Results Summary: gene selection process	Analytical Methods used
O'reilly et al,[22] 2002	Case control	20 female patients 35 to 75 y old (mean age, 51.8 y) diagnosed with detrusor instability. 20 age- and sex-matched controls 35 to 71 y old (mean age, 53.6 y) were recruited, in whom urodynamics showed a stable bladder.	Quantitative RT-PCR reveals significantly decreased P2X1, P2X4, and P2X7 expression but significantly increased P2X2 expression in idiopathic detrusor instability bladders. As only P2X2 expression is increased in idiopathic detrusor instability bladders, it strongly implies that a P2X1 or P2X2 heteromultimer is crucial for the changes in purinergic innervation in idiopathic detrusor instability bladders.	Immunohistochemistry Quantitative RT-PCR
Maake et al,[23] 2006	Case control	Detrusor samples of 13 OABs (sensory urge and detrusor instability) were obtained before botulinum toxin injection and compared with those of 8 normally contractile, no obstructed bladders obtained during radical cystectomy.	In the smooth muscle of patients with detrusor instability and sensory urge, a significant 2.4- and 2.2-fold increase, respectively, in SMTN variant 1 messenger RNA was observed compared with that of normal controls. Analyses at the SMTN protein level confirmed significant up-regulation in these bladder dysfunctions by a factor of 2.3 and 1.8, respectively. No significant difference in SMTN expression was observed between detrusor instability and sensory urge.	Northern blot Quantitative RT-PCR Immunohistochemistry
Moore et al,[24] 2001	Case control	Detrusor samples were taken from: controls, at cystectomy for cancer or cystoscopic biopsy for hematuria (n=22; age, 33–88 y), child bladder, at surgical correction of vesicoureteral reflux (n=21; age 4 mo to 2 y), and adults with detrusor instability at cystoscopy-cystodistension (n=18; age 30–81 y).	The lack of P2X3 and P2X5 may impair control of detrusor contractility and contribute to the pathophysiology of urge incontinence.	Immunohistochemistry
Schofield et al,[25] 2005	Case control	A series of 18 women with urodynamically proven detrusor instability (median age, 62 y; range, 39–85 y), who were refractory to treatment, underwent cystoscopy and cold cup biopsy. Controls (n=26; median age, 65 y) were females without urgency or urge incontinence, undergoing cystoscopy for other indications.	The increase in GAP43 with age and with previous cystitis history suggests that neuronal sprouting is important in some subsets of patients with idiopathic detrusor instability.	Immunohistochemistry
Mukerji et al,[26] 2006	Case control	Bladder specimens obtained from patients with PBS (n=16), detrusor overactivity (n=14), and asymptomatic microscopic hematuria (controls, 17).	There was marked increase of TRPM8-immunoreactive nerve fibers in IDO (P=.0249) and PBS (P<.0001) specimens, compared with controls. A significantly higher number of TRPM8-immunoreactive axons were also seen in the IDO (P=.0246) and PBS (P<.0001) groups. Urothelial TRPM8 and TRPM8-immunoreactive thick myelinated fibers seemed unchanged in IDO and PBS.	Immunohistochemistry
Roosen et al,[27] 2009	Case control	32 patients with OAB and refractory detrusor overactivity, and 8 controls without lower urinary tract symptoms underwent cystoscopic bladder biopsy.	Significant 2-fold up-regulation of cadherin-11 was found in the suburothelium of patients with OAB compared with that in controls (P=.018), whereas β-catenin was similar in the groups (P=.6).	Quantitative immunohistochemistry
Neuhaus et al,[28] 2005	Case control	Control tissue samples were taken from the bladder dome, excluding the trigonal area, at cystectomies or as biopsies during transurethral tumor resections (5 women: mean age, 65 y; SD, 10.5; range, 54–81; 6 men: 73.5 y; SD, 5.7; range, 64–80.	Semiquantitative analyses showed significantly higher Cx43 expression in the detrusor muscle and a tendency to higher Cx45 expression in the suburothelial layer associated with urge symptoms, whereas Cx40 expression was unaffected.	Indirect immunofluorescence
Roberts et al,[29] 2020	Case control	Samples from patients with no symptoms of OAB (non-OAB cohort: age, 72.0±2.3 y) and those from patients with symptoms of OAB (clinical diagnosis, frequency ≥8/d, urgency with or without urgency incontinence), diagnosed with IDO	OABs exhibited greater TRPV4-induced ATP release with age dependence. These data provide the first evidence in humans for the key functional role of TRPV4 in urothelium with specific mechanisms and identify TRPV4 up-regulation in aging and OABs	Western blotting

Reference	Study design	Study population and age (average/range)	Results Summary: gene selection process	Analytical Methods used
		(OAB with no neurologic abnormality, OAB cohort: age, 60.0± 5.0 y) were collected.		
Mukerji et al,[30] 2006	Case control	Bladder specimens were obtained from patients with detrusor overactivity (n=12) and controls with asymptomatic microscopic hematuria (n=16).	Muscarinic receptor subtypes 2 and 3 immunoreactivity significantly correlated with the urgency score (*P*=.0002 and *P*=.0206, respectively) and muscarinic receptor subtype 2 immunoreactivity correlated with the frequency score (*P*=.0029).	Immunohistochemistry

ATP, adenosine triphosphate; *GAP43*, growth-associated protein 43; *IDO*, idiopathic detrusor overactivity; *OAB*, overactive bladder; *P2X*, purinergic receptor P2X; *PBS*, painful bladder syndrome; *RT-PCR*, reverse transcriptase-polymerase chain reaction; *SMTN*, smoothelin; *TRPM8*, transient receptor potential cation channel subfamily M member 8; *TRPV4*, transient receptor potential cation channel subfamily V member 4.

TABLE 2

Genetic analysis (DAVID) of investigated significant genes

DAVID symbol	DAVID name	Results	Functions
Up-regulated genes			
P2RX2	Purinergic receptor P2X 2	Up-regulated in OAB	Nuclear inner membrane Intrinsic component of nuclear inner membrane Ligand-gated cation channel activity Nucleotide receptor activity Ligand-gated channel activity
SMTN	Smoothelin	Up-regulated in OAB	Smooth muscle contraction Contractile fiber Actin-mediated cell contraction Actin filament-based movement Actin-myosin filament sliding
GAP43	Growth-associated protein 43	Up-regulated in OAB	Transmitter-gated ion channel activity Extracellular ligand-gated ion channel activity Neuron projection organization Neurotransmitter receptor activity Modulation of chemical synaptic transmission
TRPM8	Transient receptor potential cation channel subfamily M member 8	Up-regulated in OAB	Calcium ion transport Calcium ion transmembrane transporter activity Divalent inorganic cation transmembrane transporter Antimicrobial humoral response Ligand-gated cation channel activity
CDH11	Cadherin 11	Up-regulated in OAB	Cell-cell junction organization Extrinsic component of membrane Cell-cell junction Banded collagen fibril Complex of collagen trimers
GJC1	Gap junction protein gamma 1	Up-regulated in OAB	Regulation of heart contraction Regulation of blood circulation Cell-cell signaling involved in cardiac conduction Cardiac muscle cell contraction sinoatrial node cell to atrial cardiac muscle cell communication
CHRM2	Cholinergic receptor muscarinic 2	Up-regulated in OAB	Acetylcholine receptor signaling pathway Cellular response to acetylcholine Adenylate cyclase-inhibiting G protein—coupled receptor signaling pathway Postsynaptic signal transduction Serotonin receptor activity
CHRM3	Cholinergic receptor muscarinic 2	Up-regulated in OAB	Serotonin receptor activity G protein—coupled amine receptor activity Acetylcholine receptor signaling pathway Cellular response to acetylcholine Adenylate cyclase-inhibiting G protein
TRPV4	Transient receptor potential cation channel subfamily V member 4	Down-regulated in OAB	Regulation of cytosolic calcium ion concentration Voltage-gated potassium channel activity Cellular calcium ion homeostasis Protein autophosphorylation Inorganic cations import across plasma membrane
Down-regulated genes			
P2RX3	Purinergic receptor P2X 3	Down-regulated in OAB	Nuclear inner membrane Intrinsic component of nuclear inner membrane Nucleotide receptor activity Excitatory extracellular ligand-gated ion channel activity Extracellular ligand-gated ion channel activity
P2RX5	Purinergic receptor P2X 5	Down-regulated in OAB	Extracellular ligand-gated ion channel activity Calcium ion transport into cytosol Cytosolic calcium ion transport Positive regulation of cytosolic calcium ion Response to purine-containing compound

DAVID, Database for Annotation, Visualization, and Integrated Discovery; *OAB*, overactive bladder.

TABLE 3

Top 5 clusters with their representative enriched terms (1 per cluster) via genetic analysis method

GO	Category	Description	Count	%	$Log_{10}(P)$	$Log_{10}(q)$
G0:0007268	G0 Biological Processes	Chemical synaptic transmission	6	54.55	−8.67	−4.75
G0:0006939	G0 Biological Processes	Smooth muscle contraction	4	36.36	−8.49	−4.75
G0:0044057	G0 Biological Processes	Regulation of nervous system process	5	45.45	−5.99	−2.99
G0:0008015	G0 Biological Processes	Blood circulation	4	36.36	−5.03	−2.29
G0:0009266	G0 Biological Processes	Response to temperature stimulus	3	27.27	−4.59	−1.94

"Count" is the number of genes in the user-provided lists with membership within the genetic analysis method. "%" is the percentage of all the user-provided genes that are found in the given ontology term (only input genes with at least 1 ontology term annotation are included in the calculation). "Log_{10}(P)" is the *P* value in log base 10. "Log_{10}(q)" is the multitest adjusted *P* value in log base 10.

GO, Gene Ontology.