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REVIEW ARTICLE

Genetic variants and expression changes in urgency urinary incontinence: A systematic review

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Abstract

Aim: To perform a systematic review summarizing the knowledge of genetic variants, gene, and protein expression changes in humans and animals associated with urgency urinary incontinence (UUI) and to provide an overview of the known molecular mechanisms related to UUI.

Methods: A systematic search was performed on March 2, 2020, in PubMed, Embase, Web of Science, and the Cochrane library. Retrieved studies were screened for eligibility. The risk of bias was assessed using the ROBINS-I (human) and SYRCLE (animal) tool. Data were presented in a structured manner and in the case of greater than five studies on a homogeneous outcome, a meta-analysis was performed.

Results: Altogether, a total of 10,785 records were screened of which 37 studies met the inclusion criteria. Notably, 24/37 studies scored medium-high to high on risk of bias, affecting the value of the included studies. The analysis of 70 unique genes and proteins and three genome-wide association studies showed that specific signal transduction pathways and inflammation are associated with UUI.

Abbreviations: ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CI, confidence interval; Cr, creatinine; CRP, C-reactive protein; ECM, extracellular matrix; GWAS, genome-wide association study; MCP, monocyte chemoattractant protein; M-Ras, muscarinic-Ras; NGF, nerve growth factor; OAB, overactive bladder; ROBINS-I, risk of bias in non-randomized studies of interventions; *SD*, standard deviation; *SE*, standard error; SMD, standardized mean difference; SYRCLE, SYstematic Review Centre for Laboratory animal Experimentation; TRPV1, transient receptor potential cation channel subfamily V member 1; UUI, urgency urinary incontinence.

Egbert Oosterwijk and Kirsten B. Kluivers contributed equally to this study.

A meta-analysis on the predictive value of urinary nerve growth factor (NGF) levels showed that increased urinary NGF levels correlate with UUI.

Conclusion: The collective evidence showed the involvement of two molecular mechanisms (signal transduction and inflammation) and NGF in UUI, enhancing our understanding of the pathophysiology of UUI. Unfortunately, the risk of bias was medium-high to high for most studies and the value of many observations remains unclear. Future studies should focus on elucidating how deficits in the two identified molecular mechanisms contribute to UUI and should avoid bias.

KEYWORDS

gene expression changes, genetic variants, protein expression changes, urgency urinary incontinence

1 | INTRODUCTION

Urgency urinary incontinence (UUI) is a prevalent symptom that negatively impacts the quality of life.[1,2] Patients with UUI sense a sudden, compelling desire to pass urine that is difficult to defer combined with the involuntary loss of urine.[2,3] The reported prevalence of UUI ranges between 1.8% and 30.5%, and differs substantially due to different definitions in studies.[4] Clear risk factors for UUI are age, obesity, and postmenopausal status in women.[5–8]

The pathophysiology of UUI is considered to be multifactorial: both intrinsic and environmental factors are involved.

The underlying processes that contribute to the development of UUI are still unresolved. Although the cellular and/or molecular mechanisms related to UUI have been studied, most studies focus on the overarching overactive bladder (OAB) syndrome. The current systematic review focusses on one clinically well-defined and objectively measurable symptom (UUI) to be able to study the relation between a clear phenotype and/or population and cellular/molecular mechanisms. Because several symptoms (urgency, urinary frequency, nocturia, and/or UUI) may indicate OAB, a systematic review of all these symptoms or OAB as a whole may lead to inaccuracy or cluttering in the results due to ill-defined (mixed) populations or a combination of phenotypes. This was one of the drawbacks noticed in a recent systematic review of biomarkers of several lower urinary tract symptoms (LUTS), including OAB.[9] A systematic review summarizing and critically evaluating all available evidence of cellular and/or molecular mechanisms underlying UUI is lacking. Such overview is critical to understand the mechanisms involved in the pathophysiology that leads to UUI.

1.1 | Objective

This systematic review combines and summarizes studies —both human and animal–on genetic variants, gene, and protein expression changes in relation to UUI.

2 | MATERIALS AND METHODS

We investigated human and animal studies (*domain*) on genetic variants, gene and/or protein expression changes (*outcome*) in relation to UUI (*determinant*). A prospectively registered protocol in Prospero was used concerning genetic variants, gene, and protein expression changes in relation to urinary incontinence (UI) in general (Supporting Information 1), ultimately narrowed to UUI only instead of UI in general to examine a more homogeneous population.

2.1 | Information sources and search strategy

On March 2, 2020, a systematic search was performed in PubMed, Embase, Web of Science, and the Cochrane library to identify available studies using the search strategy described in Supporting Information 2. The terms used were related to UI in general and a broad spectrum of genetic and protein expression terms and assays. References of reviews and included studies were cross-checked for studies not retrieved by the database search.

Studies were screened by two independent reviewers in two phases (title/abstract and full-text phase). Studies judged as eligible for full-text screening by one of the reviewers were screened for full text by both. The inclusion criteria were: studies with primary research data of affected cases (UUI) and controls of both humans and animals (all species and genders/sexes), examining genetic variants, gene expression,

or protein expression differences, with sufficient information to determine the risk of bias. Nocturnal enuresis in children was beyond the scope of this review and was excluded. We included studies on OAB when the criterium of incontinence was met in greater than 50% of the patients. For animal studies, a clear UUI model must be defined.

2.2 | Data extraction

The extraction of study characteristics was performed by one reviewer and verified by a second reviewer. Characteristics extracted for human studies were: first author and year of publication, gender, number of participants, definition and diagnosis-method of UUI, assessed material, assay method, investigated gene/protein/genetic variant, and results of the (value) differences between the groups (UUI and controls). For animal studies, first author and year of publication, type of animal, sex, number of subjects, UUI induction method and confirmation of diagnosis, assessed material, assay method, and investigated gene/protein/genetic variant were extracted.

The risk of bias assessment was performed by one and checked by a second reviewer. All discrepancies were discussed until agreement was reached and with the help of a third reviewer when necessary. The tools used were the Cochrane risk of bias in non-randomized studies of interventions (ROBINS-I)[10] for human studies, and the SYRCLE risk of bias tool[11] for animal studies. Differences between cases and controls in age, body mass index (BMI), and menopausal status were recorded and taken into account in the risk of bias assessment. The basic signaling questions of the ROBINS-I tool do not cover in vitro aspects of studies, that is, the derivation and preparation of cell material for outcome assessment. Therefore, seven signaling questions addressing the risk of bias for in vitro aspects of studies were used if applicable (Supporting Information 3), based on a tool developed in 2016 for in vitro studies by the National Toxicology Program.[12]

The retrieved data per outcome measure were presented in a structured manner. Outcome measures were grouped in themes according to the knowledge of their functions. Since it was expected that the outcomes of the included studies were too diverse for an overall meta-analysis, we decided that when more than five homogeneous studies explored one outcome measure, a meta-analysis of that outcome measure would be performed. When a standard error (*SE*) was provided instead of the standard deviation (*SD*) in the studies included in the meta-analysis, *SD*s were calculated using the following formula: $SD = SE \times \sqrt{n}$. Standardized mean difference (SMD) was used as an effect size measure. Subsequently, a random effect meta-analysis was performed using STATA version 15, and forest plots were created. For quantifying heterogeneity, I^2 was used.

3 | RESULTS

3.1 | Study selection

Figure 1 shows the flow chart of the review. Out of 10,785 retrieved articles, only 37 studies met the inclusion criteria and were included in the final analysis.

3.2 | Study characteristics

Tables 1 and 2 show an overview of the characteristics of the human ($N = 33$) and animal ($N = 4$) studies, respectively. Nine studies investigated genetic variants,[14–22] 4 studies gene expression changes,[23–26] and 26 studies protein expression changes[24,25,27–50] including 17 studies on urinary or serum biomarkers.[27,28,31–36,38,39,41,43–45,47,49,50] Four studies investigated possible associations in a nonhypothesis-driven manner: three genome-wide association studies (GWASs)[15,17,18] and one whole-genome expression microarray.[23] Seventy unique genes/proteins/protein-related products were analyzed in hypothesis-driven studies, and the majority of the genes/proteins/protein-related products (83%) were examined in only one study. The four animal studies were all (conditional) gene knockout mouse models.[19–22] A more extensive description of study characteristics is presented in Tables S1 and S2.

3.3 | Risk of bias of included studies

In the overall risk of bias judgment with the ROBINS-I tool, 6 studies scored high,[29,31,34,37,39,42] 18 medium-high,[15,23–25,27,28,33,35,36,38,40,41,43–46,48,50] and 9 medium-low[14,16–18,26,30,32,47,49] (Figure 2). Human studies scored poor on the topics of confounding, bias in the measurement of outcomes, and reporting of deviations between study groups. In the SYRCLE tool, all four animal studies scored unclear on risk of bias for most items (Figure 3).[19–22] The topics blinding (performance and detection), random outcome assessment, and incomplete outcome data were scored most often as contributing to unclear the risk of bias. Tables S3 and S4 show the risk of bias assessment per study.

3.4 | Synthesis of the results

The overview of the extracted results of the included studies are displayed in Tables 3 (nonhypothesis-driven)

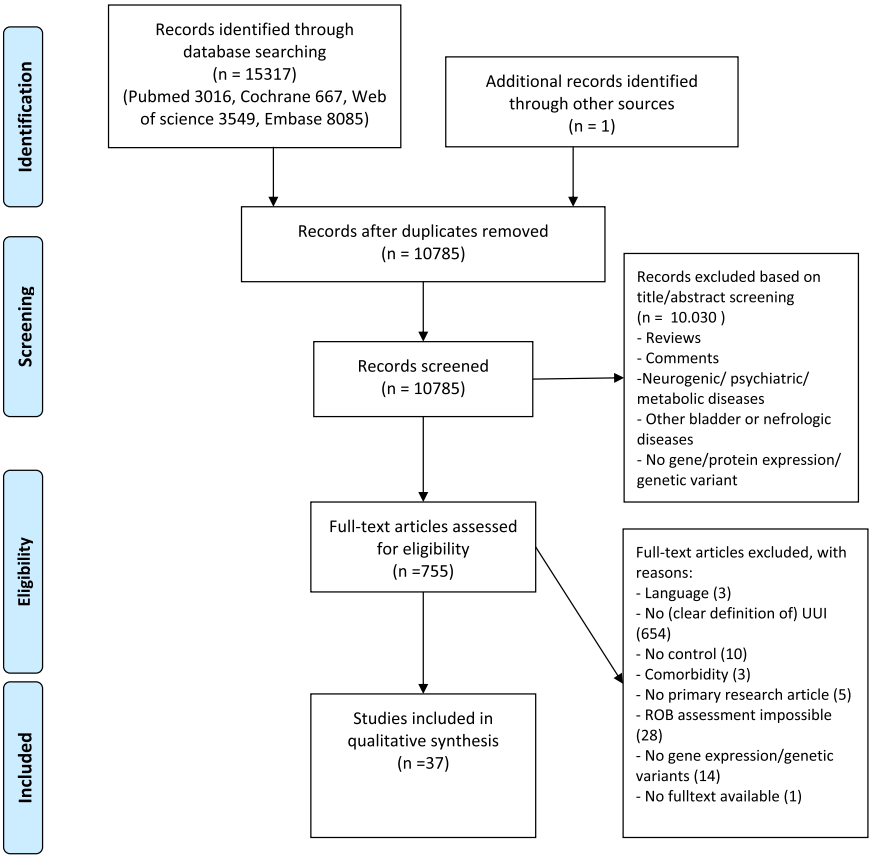

FIGURE 1 Systematic selection of articles and main reasons for exclusion based on Prisma 2009. ROB, risk of bias; UUI, urgency urinary incontinence[13]

and 4 (examining specific gene/protein/protein-related product). Concerning genetic variants, the three GWASs did not find any replicable association (Table 3).[15,17,18] Another human study, investigating specific predefined polymorphisms, indicated an association between a polymorphism in the androgen receptor and UUI.[14] The (conditional) knockout of muscarinic-Ras (M-Ras),[19] β-1 integrin,[20] and Slo1[21,22] genes led to UUI-like symptoms in animals, demonstrating a functional correlation between these genes and the occurrence of UUI.

Concerning gene expression differences, a transcriptome analysis of bladder biopsies showed several associated genes per *p*-value threshold, suggesting the involvement of multiple molecular pathways[23] (Table 3). In other studies examining gene expression, only one to three genes were examined and the results were conflicting or differences were not significant.[24–26]

The vast majority of the included studies examined protein expression(-related) differences that involved urinary or serum biomarkers (Table 4). These potential biomarkers were often tested in individual studies only and results were not independently validated. In view of the former, these single-study results are not discussed separately and can be found in Table 4.

Nerve growth factor (NGF) was the most represented biomarker (12 studies). Figure 4 shows the meta-analysis of urinary NGF/creatinine (Cr) values of patients with UUI versus controls. Two studies were not included in the meta-analysis because log-transformed data before analysis were reported[47] or only median and interquartile values were reported[43] instead of means and *SD* or *SE*. Five studies included in the meta-analysis were from one research group.[38,39,41,42,44] Communication with the corresponding author of the studies by email confirmed that there was no overlap of included subjects in these studies. Two studies[38,44] did not report which measure (*SD* or *SE*) was used. Therefore, we employed a conservative approach and assumed that the studies used *SE* and these were re-calculated into *SD*. This random effect meta-analysis showed a pooled SMD of 1.01 (confidence interval (CI) = 0.49–1.52, $I^2 = 90.0\%$; Figure 4). A sensitivity analysis, assuming the unknown measurement units were SDs, resulted in a similar pooled effect (Table S5).

Two studies examined adenosine triphosphate (ATP) release in relation to UUI. Tissue ATP levels were substantially pronounced and urinary UUI levels were significantly increased in UUI patients.[37,49] In three studies, urinary brain-derived neurotrophic factor (BDNF)/Cr levels were investigated. A trend toward higher BDNF/Cr levels was observed but a clear association between elevated urine BDNF/Cr levels and UUI could not be established.[27,28,47] For serum C-reactive protein (CRP), three out of four studies showed increased levels in UUI patients compared

TABLE 1 Study characteristics of human studies

First author (year of publication)	Assay/method	*n*, UUI	*n*, controls	Definition trait			Assessed material	Analyzed genes/proteins
				Severity	Assessment of clinical symptoms	UDT		
Alkis et al. (2017)[27]	ELISA	16	45	NI	By 3-day VD	Y	Urine	BDNF, GAG, MCP-1, and NGF
Antunes-Lopes et al. (2013)[28]	ELISA	37	20	Naïve to any form of treatment, symptoms ≥ 6 months in duration	By 7-day VD and USS	NI	Urine	BDNF, GDNF, and NGF
Birder et al. (2013)[29]	Western blot	8	7	Nonneurogenic UUI refractory to antimuscarinics: frequency > 10/day, ≥1 UUI/day	NI	NI	Primary HBUC of biopsies of posterior wall	M3R and TRPV1
Carey et al. (2000)[30]	EM/IHC	13/7	11/5	Severe idiopathic detrusor instability not specified	NI	Y	UBSM biopsies above the trigone and midline	(complementary) Dense plaques, membrane caveolae, and vinculin
Cartwright et al. (2010)[23]	Whole-genome expression microarray	5	5	Detrusor overactivity, with symptomatic urinary urgency and UUI, >10 voids/day	By 3-day VD and ICIQ-FLUTS	Y	Urothelium, lamina propria, and UBSM from biopsies of the posterior bladder wall	Whole genome
Cornu et al. (2011)[14]	DNA sequencing	30	66	UUI frequency not specified, >3 months	NI	NI	Blood	Androgen receptor CYP-17, CYP-19, and estrogen receptor-1
Christiaansen et al. (2011)[24]	RT-PCR, FACS, and ELISA	3	3	Urinary frequency > 10/day, ≥1 UUI/day	NI	NI	HBUC of random biopsies	HIF-1a, HIF-2a, and VEGF
Chuang et al. (2010)[31]	High-sensitivity CRP assay	18	20	UUI ≥ 1/day	NI	NI	Serum and urine	CRP
Farhan et al. (2019)[32]	ELISA	18	10	UUI ≥ 1/day	By 3-day VD	NI	Urine	MCP-1
Funada et al. (2018)[15]	GWAS	187	4096	UUI (OABSS ≥ 3, urgency score ≥ 2, UUI score > 2 in OABSS)	By OABSS	NI	Blood	GWAS with 99,059 SNPs, and additionally three genes previously associated with UUI (ADAMTS16, CIT, and ZNF521)

(Continues)

TABLE 1 (Continued)

First author (year of publication)	Assay/method	*n*, UUI	*n*, controls	Definition trait			Assessed material	Analyzed genes/proteins
				Severity	Assessment of clinical symptoms	UDT		
Honda et al. (2014)[16]	PCR-based	61	100	UUI ≥ 1/day	By 3-day VD	NI	Hair	1 Variant in B3-AR
Hsiao et al. (2012)[33]	Particle-enhanced turbidimetric assay	39	18	UUI ≥ 1/3 days	By 3-day VD, OABSS, and modified IUSS	Y	Serum	CRP
Keske et al. (2019)[34]	Colorimetric assays	38	29	NI	NI	Y	Serum	TAC, TOS, PON, arylesterase, AOPP, and IMA
Kim et al. (2015)[35]	ELISA	39	62	≥3 UUI/3 days, no history of diagnosis/ treatment for OAB	By 3-day VD	Y	Urine	HB-EGF and NGF
Kubota et al. (2018)[36]	ELISA	612	147	NI	By OABSS	NI	Urine	Stem cell factor
Kumar et al. (2010)[37]	Luminometry	8	9	Refractory symptoms of UUI, frequency, and urgency, not further specified	NI	Y	Urothelium. Patients: bladder dome, Controls: site distant from tumor (nonirradiated bladder), normal looking bladder area	ATP
Kuo et al. (2010)[38]	ELISA	25	28	≥1 UUI/3 days	By 3-day VD	Y	Urine	NGF
Kuo et al. (2010)[39]	ELISA	22	49	≥1 UUI/3 days	By 3-day VD	Y	Urine	NGF
Li et al. (2011)[25]	Immunofluorescence, PCR, and Western blot	2	2	Nonneurogenic UUI refractory to antimuscarinics: frequency > 10/day, ≥1 UUI/day	NI	NI	HBUC	TRPV1
Li et al. (2013)[40]	Immunohisto-fluorescence and HPLC	4	6	≥1 UUI/day, frequency > 10/day	NI	NI	HBUC	Polyamines
Liu et al. (2007)[26]	Quantitative competitive RT-PCR	12	42	Refractory UUI, frequency, urgency, and nocturia, despite ≥ 2	NI	Y	Urothelium, lamina propria, and UBSM biopsies from body 2 cm from the left ureteric	TRPV1

TABLE 1 (Continued)

First author (year of publication)	Assay/method	*n*, UUI	*n*, controls	Definition trait			Assessed material	Analyzed genes/proteins
				Severity	Assessment of clinical symptoms	UDT		
				anticholinergics and bladder training >1 year			orifice and central trigone	
Liu et al. (2008)[41]	ELISA	80	40	≥1UUI/day, urgency and frequency	By 3-day VD	Y	Urine	NGF
Liu et al. (2010)[42]	IHC and ELISA	18	14	UUI patients who underwent botulinum toxin A injection	NI	Y	Urothelium (location unknown) and urine	NGF
Liu et al. (2011)[43]	ELISA	17	31	≥3 UUI/3 days, refractory to 3 months of treatment	By 3-day VD	Y	Serum and urine	NGF
Liu et al. (2011)[44]	ELISA	106	84	≥1 UUI/3 days	By 3-day VD	NI	Urine	NGF
Liu et al. (2013)[45]	Bead-based human serum adipokine panel B kit and particle-enhanced turbidimetric assay	14	26	≥3 UUI/3 days, refractory to previous antimuscarinic therapy	By 3-day VD	NI	Serum	CRP, IL-1b, IL-6, IL-8, insulin, leptin, MCP-1, NGF, and TNF- a
Moore et al. (2001)[46]	IHC	18	22	UUI refractory to antimuscarinic drugs for > 12 months	NI	Y	Bladder biopsy tissue including UBSM cells	P2X(1–7)
Penney et al. (2019)[17]	GWAS	1942	4811	UUI weekly	Biennial questionnaire	NI	Blood and/or cheek cell sample	GWAS of 1,410,640 variants
Richter et al. (2015)[18]	GWAS and replication in a second cohort	1102	405	Anamnestic symptoms of UUI, >1/month who leaked sufficiently to wet or soak their underpants or clothes	NI	NI	Blood	GWAS of 975,508 variants, after imputation 9,077,347
Richter et al. (2017)[47]	ELISA and magnetic polystyrene bead-based immunoassay	260	54	Refractory UUI of ≥ 6/3 days, despite ≥ 1 supervised behavioral/ physical therapy and ≥ 2 anticholinergic drugs	By 3-day VD	Y	Urine	BDNF, CGRP, collagenase activity, GMC-SF, IL-Ib, IL-6, IL-8, MMP-1, MMP-2, MMP-9, NGF, NTx, TNF-a, tropoelastin, and substance P

(Continues)

TABLE 1 (Continued)

First author (year of publication)	Assay/method	*n*, UUI	*n*, controls	Definition trait			Assessed material	Analyzed genes/proteins
				Severity	Assessment of clinical symptoms	UDT		
Schofield et al. (2005)[48]	IHC	18	23	Refractory UUI, persistent disabling urgency of ≥ 8 voids/24 h, despite ≥2 anticholinergic drugs > 12 months	By 3-day VD	Y	Subepithelial and UBSM nerve fibers	GAP-43
Silva-Ramos et al. (2013)[49]	Luciferin–luciferase bioluminescence assay and ELISA	34	36	UUI ≥ 1/day, urgency, frequency ≥ 8 voids/day	NI	Y	Urine	ATP and NGF
Ustundag et al. (2019)[50]	Commercially available kits, immunoassay, and nephelometry	42	34	OAB-questionnaire score > 11	By OAB-questionnaire	NI	Serum	Calcium, triglyceride, HDL, LDL, total cholesterol, Hba1c, parathormone, vitamin D CRP, ferric reducing power of plasma, albumin, IMA, native thiol, total thiol, and disulfide

Abbreviations: ATP, adenosine triphosphate; B1 integrin, β1 integrin; B3-AR, Beta-3 adrenergic receptor; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; CRP, C-reactive protein; ELISA, enzyme-linked immuno sorbent assay; FACS, fluorescence-activated cell sorting; GAG, glycosaminoglycans; GAP-43, growth association protein 43; GDNF, glial cell line-derived neurotrophic factor; GMC-SF, granulocyte-macrophage colony-stimulating factor; GWAS, genome-wide association study; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HBUC, human bladder urothelium cells; HDL, high-density lipoprotein; HIF, hypoxia-inducible factor; HPLC, high-performance liquid chromatography; ICIQ-FLUTS, International Consultation on Incontinence Questionnaire-Female Lower Urinary Tract Symptoms; IHC, immunohistochemistry; IL, interleukin; IUSS, Indevus Urgency Severity Score; LDL, low-density lipoprotein; M2/3R, muscarinic 2/3 receptor; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; M-Ras, muscarinic-Ras; NGF, nerve growth factor; NI, no information; NTx, N-terminal telopeptide type 1 collagen; OAB, overactive bladder; OABSS overactive bladder symptom score; SNP, single-nucleotide polymorphism; TNF-α, tumor necrosis factor α; TRPV1, transient receptor potential cation channel subfamily V member 1; UBSM, urinary bladder smooth muscle; UDT, urodynamic test; UUI, urgency urinary incontinence; VD, voiding diary; VEGF, vascular endothelial growth factor; Y, yes.

TABLE 2 Study characteristics of animal studies ([conditional] gene deletion mouse models)

First author (year of publication)	Animal characteristics	*n*, UUI	*n*, controls	UUI diagnosis	(c)KO	Analyzed tissue	Analyzed genes/ proteins
Ehrhardt et al. (2015)[19]	Gene deletion: KO mice backcrossed with C57Bl/6 till F10 WT: C57Bl/6 mice	PAM	PAM	Increased amplitudes of spontaneous bladder contractions, increased number of urine spots	KO	Whole animal, bladder special focus on UBSM	M-Ras, M2R, and M3R
Kanasaki et al. (2013)[20]	16 Gene deletion: B1-integrin floxed/floxed B6; 129-Itgb1tm1EfuJ mice expressing Cre WT: B1-integrin floxed/floxed B6; 129-Itgb1tm1EfuJ not expressing Cre	PAM	PAM	Dramatic loss of voiding control, that is, inability to restrict voiding location and distribution of spot sizes	cKO	Whole bladder, special focus on urothelium	B1-integrin
Meredith et al. (2004)[21]	Gene deletion: KO C57Bl/6 mice WT: C57Bl/6 mice	PAM	PAM	Many small urination spots, yellow perineal staining	KO	Whole bladder, special focus on UBSM	BK channel and Slo1
Thorneloe et al. (2005)[22]	Gene deletion: $Slo^{-/-}$ mice not further specified WT: $Slo^{+/+}$ mice not further specified	8	8	Increased bladder pressures, increased frequency of pressure oscillations, and urine leakage	KO	UBSM stripes	BK channel and Slo1

Abbreviations: cKO, conditional knockout; KO, knockout; PAM, per assay mentioned; UBSM, urinary bladder smooth muscle; UUI, urgency urinary incontinence; WT, wildtype.

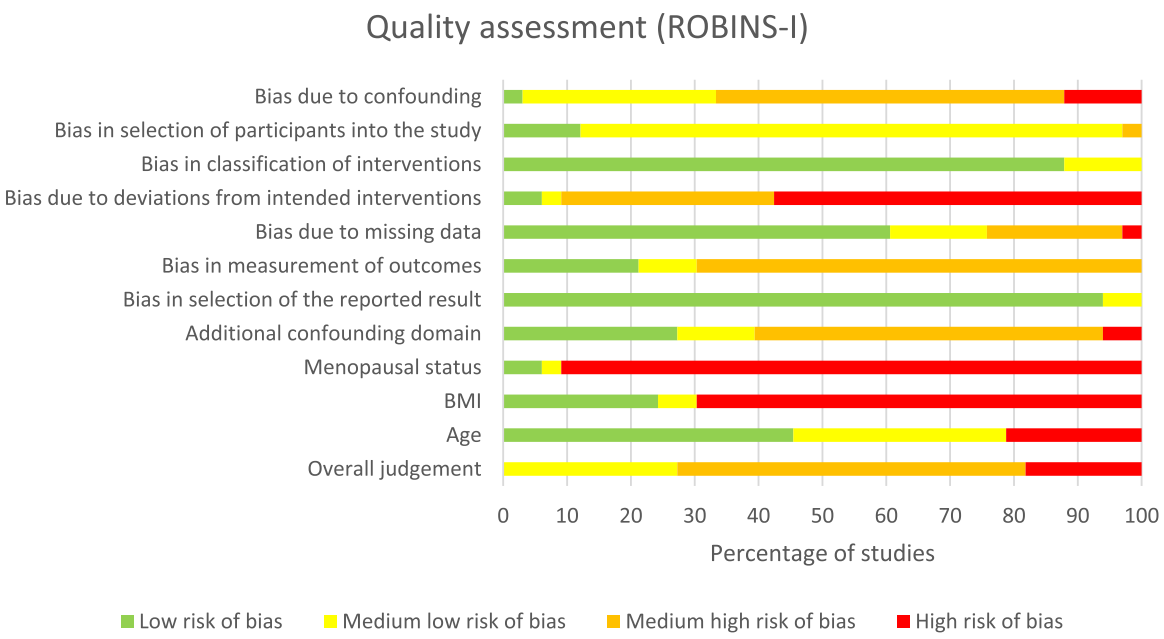

FIGURE 2 Risk of bias graph of each item from the Cochrane ROBINS-I tool that was applied to all included human studies and scored by two investigators. For each item, several questions were scored with answers ranging from yes/probably yes/probably no/no/no information/not applicable. Finally, all items were scored as low risk of bias, medium-low risk of bias, medium-high risk of bias, and high risk of bias. BMI, body mass index; ROBINS-I, risk of bias in non-randomized studies of interventions

with controls, one study failed to demonstrate this association.[31,33,45,50] In two studies examining ischemia modified albumin (IMA) significantly elevated IMA levels were found in UUI patients compared with controls in one study,[32] but this was not confirmed in the second study.[36]

Two studies examining urinary[47] or serum[45] levels of interleukin-1B, -6, and -8 showed increased levels in the serum of UUI patients but no significant differences in the urine. Finally, in three studies urinary[27,32] and serum[45] levels of monocyte chemoattractant protein-1 (MCP-1) were

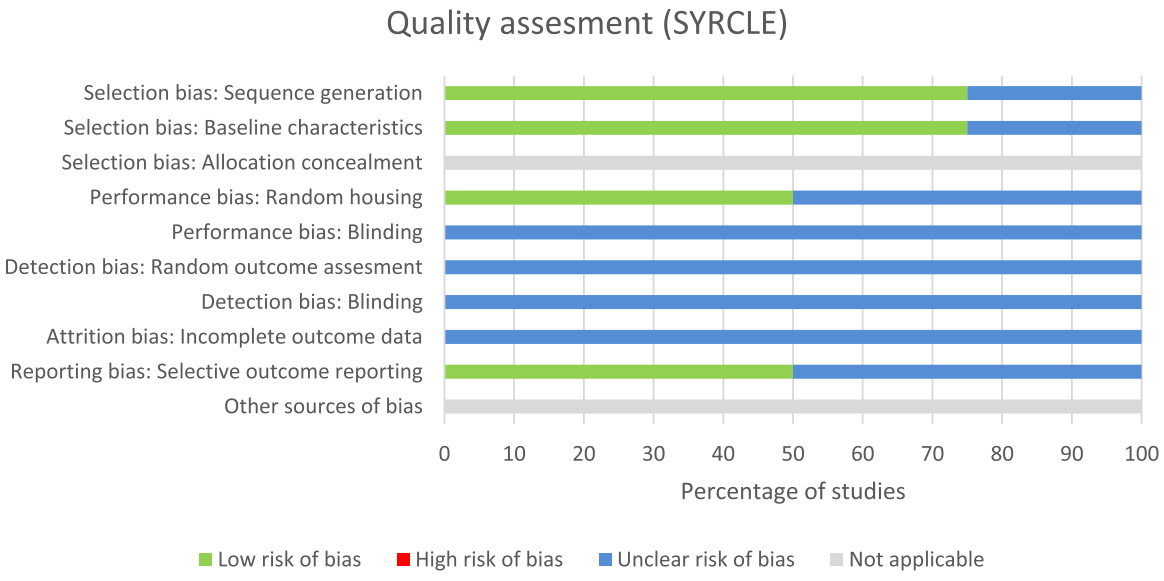

FIGURE 3 Risk of bias graph of each item from the SYRCLE tool that was applied to all included animal studies and scored by two independent investigators. For each item, "yes" correlates to low risk of bias and scores 1, while "no" correlates to high risk of bias and scores 0. SYRCLE, SYstematic Review Centre for Laboratory animal Experimentation

TABLE 3 Results of nonhypothesis-driven studies

First author (year of publication)	Type of study	Population studied	Results		Extra
Cartwright et al. (2010)[23]	Transcriptome analysis of bladder biopsies (Affymetrix array)	Women with and without UUI	≥Twofold change, $p < .005$: *FAM69C, MYOM2, SLC5A9, C3orf16, RUNX1, GAN, PWRN1, PDE5A, NCAM1, LOC100126784, MYLK4, GFRA3, SPTBN1, S100B, PTGS1, RGS11, MYOM1, PLN, NRP2, FXYD7, RYR2, MAEL, DCAF12L1*, and *CHRM3* $p < .01$: 1115 Differentially expressed genes		Pathway analysis: cytoskeleton remodeling, cell adhesion, smooth muscle contraction, cholinergic, G-protein coupled, and calcium-dependent signaling
Richter et al. (2015)[18]	Two-stage GWAS	Postmenopausal women with or without UUI	Discovery cohort: 17 genetic variants: *CIT*-gene, *SLC16A7*, and intergenic ($p = 4.57–9.32 \times 10^{-7}$)	Replication cohort: replication failed	Meta-analysis of both cohorts: 17 genetic variants: (15 new) 5p15 (*ADAMTS16*), 10p12 (*LINC01516*), 11q14 (intergenic), 12p11 (intergenic), 12q24 (*CIT* gene), and 18q11 (*ZNF521*; $p = 1.91–9.47 \times 10^{-7}$)
Funada et al. (2018)[15]	Two-stage GWAS	General population with or without UUI	Discovery cohort: rs4467538 ($p = 8.47 \times 10^{-8}$)	Replication cohort: replication failed	Checked for associations between UUI and *ADAMTS16*, *CIT*, and *ZNF521*, replication failed
Penney et al. (2019)[17]	GWAS	Nurse participants with or without UUI	No genome-wide significant associations		NA

Abbreviations: GWAS, genome-wide association study; NA, not applicable; UUI, urgency urinary incontinence.

TABLE 4 Results of studies researching specific genes/proteins/product

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	*p*	Unit
AIP	Ustundag et al. (2019)[50]	Serum	0.048 ± 0.31	0.046 ± 0.26	.982	NI
Albumin	Ustundag et al. (2019)[50]	Serum	46 ± 10	55 ± 17	.151	g/L
Androgen receptor	Cornu et al. (2011)[14]	Blood	AR polymorphism (combination of two alleles containing more than 21 CAG repeats) is significantly associated with UUI		.02	NA
AOPP	Keske et al. (2019)[34]	Serum	134.4 ± 32.6	138.9 ± 46.0	.641	NI
Arylesterase	Keske et al. (2019)[34]	Serum	184.6 ± 39.2	189.7 ± 55.7	.662	NI
ATP	Kumar et al. (2010)[37]	Urothelium	1064.2 ± 238.9	45.7 ± 4.9	NI	pmol/g
	Silva-Ramos et al. (2013)[49]	Urine	27.5 ± 8.3	7.2 ± 1.7	.022	pM
B1-integrin	Kanasaki et al. (2013)[20]	Mutated mice	B1-KO mice exhibited UUI phenotype compared with controls. Urine spot number and spot area as a percentage of the filter paper area were significantly greater in the B1-cKO mice; frequency distribution of urine spot volumes showed B1-cKO mice had a greater proportion of urine deposits that were moderately large			
B3-AR	Honda et al. (2014)[16]	Hair	Significantly higher frequency of variant Trp64Arg/Arg64Arg I in B3-AR n OAB group versus controls. Within OAB-group no significant difference in UUI pts with and without variant			
BDNF	Alkis et al. (2017)[27]	Urine	844.3 ± 286.3	340.2 ± 199.0	NI	pg/mg
	Antunes-Lopes et al. (2013)[28]	Urine	628.1 ± 590.5	110.4 ± 159.5	NI	pg/mg
	Richter et al. (2017)[c,47]	Urine	62.0 ± 1.4	46.3 ± 1.2	NS difference	pg/mg
Calcium	Ustundag et al. (2019)[50]	Serum	Median [IQR]: 2.37 [0.12]	2.39 [0.07]	.724	mmol/L
CGRP	Richter et al. (2017)[c,47]	Urine	595.5 ± 1.3	527.4 ± 1.5	NS difference	pg/mg
Cholesterol, total	Ustundag et al. (2019)[50]	Serum	5.58 ± 1.08	6.05 ± 1.13	.071	mmol/L
Collagenase I activity	Richter et al. (2017)[c,47]	Urine	279.2 ± 449.0	138.9 ± 321.2	NS difference	μg/min/mg

TABLE 4 (Continued)

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	p	Unit
CRP	Chuang et al. (2010)[31]	Serum	2.96 ± 0.47	0.93 ± 0.27	.0002	mg/L
		Urine	All samples below assay sensitivity			
	Hsiao et al. (2012)[33]	Serum	Median [IQR]: 0.12 [0.03–0.26]	0.055 [0.04–0.08]	.032	mg/dl
	Liu et al. (2013)[45]	Serum	0.33 ± 0.37	0.06 ± 0 0.04	.011	pg/ml
	Ustundag et al. (2019)[50]	Serum	Median [IQR]: 29.5 [0.9]	29.5 [0.9]	.994	nmol/L
(complementary) Dense plaques	Carey et al. (2000)[30]	Detrusor muscle	No apparent differences between the groups			
CYP-17 and CYP-19	Cornu et al. (2011)[14]	Blood	No significant difference in prevalence of polymorphisms between the groups			
Estrogen receptor-1	Cornu et al. (2011)[14]	Blood	No significant difference in prevalence of polymorphisms between the groups			
Disulfide	Ustundag et al. (2019)[50]	Serum	17.0 ± 4.2	19.0 ± 6.2	.118	μmol/L
FRAP	Ustundag et al. (2019)[50]	Serum	1135 ± 283	1120 ± 264	.842	μmol/L
GAG	Alkis et al. (2017)[27]	Urine	126.2 ± 45.1	90.9 ± 60.3	NI	pg/mg
GAP-43	Schofield et al. (2005)[48]	PNT			NS difference	Area
GDNF	Antunes-Lopes et al. (2013)[28]	Urine	958.1 ± 826.2	1.220.5 ± 513.5	.128	pg/mg
GMC-SF	Richter et al. (2017)[c,47]	Urine	All samples below assay sensitivity			
Hba1c	Ustundag et al. (2019)[50]	Serum	Median [IQR]: 5.8 [0.5]	5.9 [0.7]	.363	%
HB-EGF	Kim et al. (2015)[35]	Urine	9.4 ± 7.73	4.45 ± 2.93	NI	pg/mg
HDL	Ustundag et al. (2019)[50]	Serum	1.42 ± 0.43	1.42 ± 0.38	.835	mmol/L
HIF-(1a/2a)	Christiaansen et al. (2011)[24]	HBUC	HIF-1a: 20.06 ± 11.74% HIF2-a: 13.7 ± 1.75%	HIF-1a: 21.58 ± 12.39% HIF-2a: 16.3 ± 3.15%	NS difference	%

(Continues)

TABLE 4 (Continued)

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	*p*	Unit
IMA	Keske et al. (2019)[34]	Serum	0.614 ± 0.106	0.530 ± 0.117	.003	NI
	Ustundag et al. (2019)[50]	Serum	0.629 ± 0.257	0.569 ± 0.219	.335	Absorbance unit
Insulin	Liu et al. (2013)[45]	Serum	771.58 ± 502.54	759.8 ± 471.7	.922	pg/ml
Interleukins (IL-1B, IL-6, IL-8)	Liu et al. (2013)[45]	Serum	IL-Iβ: 4.68 ± 3.10	IL-Iβ: 1.64 ± 2.37	.045	pg/ml
			IL-6: 5.78 ± 9.97	IL-6: 0.79 ± 1.05	.000	
			IL-8: 4.12 ± 3.81	IL-8: 1.45 ± 1.06	.000	
Richter et al. (2017)[c,47]	Urine	IL-6: 2.5 ± 1.5	IL-6: 3.0 ± 1.1	NS difference	pg/mg	
		IL-8: 38.4 ± 1.1	IL-8: 37.2 ± 1.3	NS difference		
			IL-1B below assay sensitivity			
LDL	Ustundag et al. (2019)[50]	Serum	3.36 ± 1.00	3.7 ± 0.98	.089	mmol/L
Leptin	Liu et al. (2013)[45]	Serum	10,942 ± 14,338	6242 ± 4038	.922	pg/ml
MCP-1	Alkis et al. (2017)[27]	Urine	635.7 ± 284.2	155.8 ± 79.4	NI	pg/mg
	Farhan et al. (2019)[32]	Urine	Mean: 209.25 ± (SEM) 30.5	48.02 ± 9	.001 (ANOVA control-wet-dry)	pg/mg
	Liu et al. (2013)[45]	Serum	132.46 ± 18.00	104.81 ± 37.39	.067	pg/ml
Membrane caveolae	Carey et al. (2000)[30]	Detrusor muscle	No apparent differences between the groups			
MMP(-1/2/9)	Richter et al. (2017)[c,47]	Urine	MMP-2: 251.8 ± 1.3	MMP-2: 183.8 ± 1.5	NS difference	pg/mg
			MMP-9: 32.8 ± 1.9	MMP-9: 28.2 ± 2.1	NS difference	ng/mg
			MMP-1 below assay sensitivity			
Muscarinic 2/3 receptor/M-Ras	Birder et al. (2013)[29]	HBUC	Nonsignificant decrease of M3R-expression in UUI group (shown in figure, exact data not shown)			
	Ehrhardt et al. (2015)[19]	Mutated mice	M-Ras$^{-/-}$ male mice exhibited UUI phenotype. Dysregulation of M2R and M3R in M-Ras$^{-/-}$ mice; male mice had a higher expression of M2R, female mice lower expression M3R. Significantly more urine spots produced by M-Ras$^{-/-}$ males compared with WT males ($p = .0124$), while M-Ras$^{-/-}$ and WT females produced similar numbers of spots			
NGF	Alkis et al. (2017)[27]	Urine	1107 ± 602.5	202.9 ± 48.4	NI	pg/mg
	Antunes-Lopes et al. (2013)[28]	Urine	488.5 ± 591.8	188.3 ± 290.2	.005	pg/mg

TABLE 4 (Continued)

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	*p*	Unit
	Kim et al. (2015)[35]	Urine	1.26 ± 1.07	0.5 ± 0.29	<.001	pg/mg
	Kuo et al. (2010)[38]	Urine	1.66 ± 3.30	0.09 ± 0.22	.015	pg/mg
	Kuo et al. (2010)[39]	Urine	1.83 ± 0.74	0.05 ± 0.02	.012	pg/mg
	Liu et al. (2008)[41]	Urine	1.7 ± 0.26	0.041 ± 0.026	.000	pg/mg
	Liu et al. (2010)[42]	Urine	0.78 ± 1.26	0.01 ± 0.02	.02	pg/mg
		Urothelium	125.87 ± 21.79	135.60 ± 13.50	.142	pg/mg
	Liu et al. (2011)[43]	Serum	Median [IQR]: 0.0 [0–33.6]	0.0728 [0–0.234]	NI	pg/ml
		Urine	Median [IQR]: 0.82 [0.13–1.84]	0.005 [0–0.028]	NI	pg/mg
	Liu et al. (2011)[44]	Urine	2.13 ± 3.87	0.07 ± 0.21	.000 (ANOVA (control-dry-wet)	pg/mg
	Liu et al. (2013)[45]	Serum	3.66 ± 2.45	2.57 ± 0.88	.045	pg/ml
	Richter et al. (2017)[c,47]	Urine	6.4 ± 1.5	5.0 ± 1.5	NS difference	pg/mg
	Silva-Ramos et al. (2013)[49]	Urine	109.5 ± 29.0	64.0 ± 13.6	.162	pg/mg
NTx	Richter et al. (2017)[c,47]	Urine	31.4 ± 1.3	15.6 ± 2.1	<.001	nM/mM
P2X(1–7)	Moore et al. (2001)[46]	PNT	P2X3 and P2X5: 0% and 0%	P2x3 and P2X5 94 and 91%	NI	%
			P2X4, P2X6, and P2X7: 36%, 33%, and 67%	P2X4, P2X6, P2X7: 16%, 18%, and 6%	<.0001 in all	
			P2X1 and P2X2: 96% and 99%	P2X1 and P2X2: 97% and 99%	0.32 and 0.16	
Parathormone	Ustundag et al. (2019)[50]	Serum	Median [IQR]: 6.68 [4.0]	6.15 [3.9]	0.715	pmol/L
Polyamines	Li et al. (2013)[40]	Urothelium	Putrescine: 0.50 ± 0.15	Putrescine: 0.16 ± 0.03	<.05	nmol/mg
			Spermidine: 2.4 ± 0.21	Spermidine: 1.0 ± 0.13	<.01	nmol/mg
			Spermine: 1.9 ± 0.27	Spermine: 0.86 ± 0.26	<.05	nmol/mg
PON	Keske et al. (2019)[34]	Serum	Median [IQR]: 144.1 [91.6–249.5]	158.6 [91.1–280.8]	.934	NI

(Continues)

TABLE 4 (Continued)

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	p	Unit
Slo	Meredith et al. (2004)[21]	Mutated mice	$Slo^{-/-}$ mice exhibited UUI phenotype compared with controls			
	Thorneloe et al. (2005)[22]	Mutated mice	$Slo^{-/-}$ mice exhibited UUI phenotype compared with controls			
Stem cell factor	Kubota et al. (2018)[36]	Urine	Median [IQR]: 1.30 [0.56–2.71]	0.26 [0.13–0.43]	<.0001	pg/mg
Substance P	Richter et al. (2017)[c,47]	Urine	257.5 ± 0.9	271.5 ± 1.1	NS difference	pg/mg
TAC	Keske et al. (2019)[34]	Serum	1.8 ± 0.199	2.1 ± 0.216	<.001	NI
Thiol, native	Ustundag et al. (2019)[50]	Serum	331 ± 64	356 ± 73	.156	μmol/L
Thiol, total	Ustundag et al. (2019)[50]	Serum	365 ± 65	394 ± 70	.095	μmol/L
TNF-α	Liu et al. (2013)[45]	Serum	3.30 ± 2.60	0.91 ± 0.84	.000	pg/ml
	Richter et al. (2017)[c,47]	Urine	All below assay sensitivity			pg/ml
TOS	Keske et al. (2019)[34]	Serum	4.7 ± 1.77	4.1 ± 1.46	.109	NI
Triglyceride	Ustundag et al. (2019)[50]	Serum	1.84 ± 1.08	1.86 ± 1.12	.927	mmol/L
Tropoelastin	Richter et al. (2017)[c,47]	Urine	17.1 ± 0.9	9.6 ± 1.2	.001	mg/mg
TRPV1	Birder et al. (2013)[29]	HBUC	Statistically significant higher receptor expression in UUI versus controls (shown in figure, exact data not shown)			
	Li et al. (2011)[25]	Urothelium	0.25 ± 0.005	0.125 ± 0.01	<.05	Mean density ratio
	Liu et al. (2007)[26]	Urothelium	Bladder body median [IQR]: 11.4 [6.7–16.1]	14.2 [8.2–20.7]	NS difference	10^5 Copies/μg
			Trigonum median [IQR]: 10.9 [8.5–15.7]	4.1 [0.77–26.2]		Total RNA
VEGF	Christiaansen et al. (2011)[24]	HBUC	23.51 ± 9.88%	24.52 ± 4.68%	NS difference	%

TABLE 4 (Continued)

Analyzed gene/protein/product	Study	Tissue[a]	Result[b] UUI	Control	*p*	Unit
Vinculin	Carey et al. (2000)[30]	Detrusor muscle	No apparent differences between the groups			
Vitamin D	Ustundag et al. (2019)[50]	Serum	Median [IQR]: 27.0 [27.5]	33.7 [30.7]	.081	nmol/L

[a]All urinary values are adjusted to urinary creatinine levels.

[b]Data are presented as means ± *SD* unless otherwise described.

[c]Data log-transformed before analysis.

Abbreviations: AIP, atherogenic index of plasma; ANOVA, analysis of variance; AOPP, advanced oxidation protein products; ATP, adenosine triphosphate; B1 integrin, β1 integrin; B3-AR, β-3 adrenergic receptor; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; CRP, C-reactive protein; FRAP, ferric reducing power of plasma; GAG, glycosaminoglycans; GAP-43, growth association protein 43; GDNF, glial cell line-derived neurotrophic factor; GMC-SF, granulocyte-macrophage colony-stimulating factor; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HBUC, human bladder urothelium cells; HDL, high-density lipoprotein; HIF, hypoxia-inducible factor; IL, interleukin; IMA, ischemia modified albumin; IQR, interquartile ratio; LDL, low-density lipoprotein; M2/3R, muscarinic 2/3 receptor; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; M-Ras, muscarinic-Ras; NA, not applicable; NGF, nerve growth factor; NI, no information; NTx, N-terminal telopeptide type 1 collagen; PNT, parasympathic nerve tissue; PON, paraoxonase; TAC, total antioxidant capacity; TNF-α, tumor necrosis factor α; TOS, total oxidant status; TRPV1, transient receptor potential cation channel subfamily V member 1; VEGF, vascular endothelial growth fac.

investigated, showing a trend toward elevated levels in UUI patients compared with controls but a statistically significant difference was achieved in only one study.[32]

4 | DISCUSSION

In search of molecular pathways involved in UUI, we performed a systematic review of literature concerning genetic variants, and differences in gene and protein expression in UUI subjects when compared with controls. The symptom of UUI was selected to examine a clear and clinically well-defined phenotype, with the aim to avoid cluttering of results. After the extended search, only 0.03% of initial studies were ultimately included in the analysis. In general, the risk of bias was judged as medium-high or high (unclear in animal studies), and the majority of the outcomes were only examined by single studies. Despite the heterogeneity between the studies—which made it a challenge to find common denominators—two major molecular themes were distinguished as being associated with UUI: *signal transduction* and *inflammation*.

4.1 | Signal transduction

Several studies suggested an association of genetic polymorphisms with UUI.[14,18] Polymorphisms in the genes encoding CIT (associated with cytokinesis), the transcription factor ZNF5521, and the androgen receptor were described, but unfortunately, the association could not be validated in independent cohorts. Although this suggests that these genes are not linked to UUI, replication in larger cohorts is necessary to draw firm conclusions. The four animal gene knockout studies all showed an association of specific gene expression with a UUI phenotype. Slo1, investigated in two studies, encodes for the pore-forming subunit of the calcium-activated BK potassium channel in bladder smooth muscle cells.[21,22] It contributes to the control and regulation of spontaneous bladder contractions by regulating its membrane potential and repolarizing the action potentials and deletion of this gene resulted in a UUI phenotype.[51,52] The studies firmly demonstrated a direct relation between Slo1 expression and regulation of bladder contractions and UUI. Whether Slo1 expression or expression levels are involved in human UUI is, however, still unsolved. Male M-Ras$^{-/-}$ knockout mice developed an UUI phenotype but the female M-Ras$^{-/-}$ mice did not.[19] The sex-dependent variation of this phenotype and the expression of both M3R and M2R has

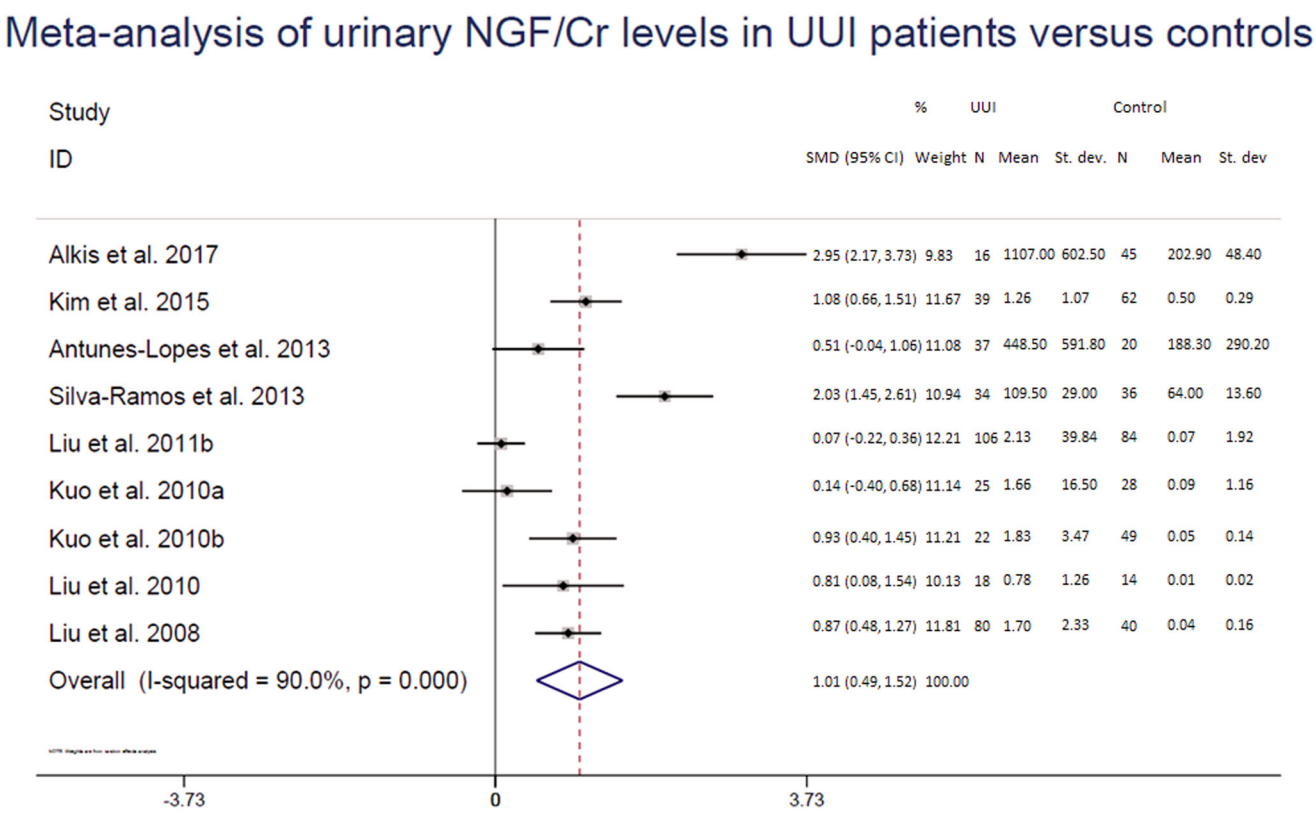

FIGURE 4 Meta-analysis of urinary NGF/Cr levels in patients with urgency urinary incontinence versus controls.
CI; confidence interval; Cr, creatinine; NGF, nerve growth factor; SMD, standardized mean difference; st. dev., standard deviation; UUI, urgency urinary incontinence

not been explained. M-Ras is expressed predominantly in fibroblasts and skeletal muscle cells and activates a wide variety of proteins. The results suggest that phenotypic changes in these cells contribute to UUI. Finally, the association of B1-integrin (encoded by the ITGB1 gene), a receptor for collagen, with UUI suggests that an aberrant extracellular matrix (ECM) composition may play a role in UUI.[20]

Concerning gene expression studies, the transcriptome analysis comparing normal versus UUI-derived bladder tissue showed the differential expression of a large number of genes linked to multiple pathways.[23] Interestingly, smooth muscle contraction, cholinergic, G-protein coupled, and calcium-dependent signaling were major pathways in which genes that are differentially expressed in UUI are involved.

Collectively, these studies show that the occurrence of aberrant signaling, abnormal responses of contractile cells, and aberrant ECM composition are intimately associated with UUI.

Finally, protein expression studies support the association of signal transduction pathways and UUI. Multiple studies demonstrated a correlation between UUI and an increased urinary NGF/Cr ratio (pooled SMD: 1.01, CI: 0.49–1.52; I^2: 90.0%). An SMD of zero denotes no effect. Nevertheless, this result should be interpreted with caution because the heterogeneity between studies was high in the participants enrolled (difference in the severity of UUI, medication use, and/or age), in the method of collection of the urinary sample, and in the method of NGF/Cr determination. It is intriguing that NGF is associated with neurological effects[53] and the collective evidence suggests that NGF also affects the bladder.[54,55] Kashyap et al.[56] showed that, when inducing bladder overactivity with acetic acid in female Sprague-Dawley rats, NGF overexpression and chemokine upregulation occurred. How this relates to UUI and whether this leads to NGF signaling remains undetermined and deserves further investigation. Importantly, elevated NGF/Cr values are not solely restricted to UUI, as these were also found to be elevated in OAB in general[57–59] and bladder pains syndrome/interstitial cystitis,[60] a conclusion recently confirmed by Siddiqui et al.[9] who reviewed biomarkers related to LUTS. In line with our findings, they judged the included studies as being of poor quality. The studies investigating urinary BDNF/Cr levels in UUI patients were inconclusive. However, transgenic animals overexpressing BDNF in the bladder showed changes in the bladder neurons leading to detrusor overactivity, a common finding in UUI.[61] This does suggest a role for

BDNF in UUI, albeit that apparently this may not be related to BDNF levels but to downstream signaling. Combined with the findings on NGF, it could conceivably be hypothesized that UUI is associated with neuronal changes and/or aberrant signaling.

Multiple studies investigated the possible involvement of the purinergic signaling pathway in UUI, showing changes in purinergic receptor (subtypes 3–7) expression,[46] ATP release,[37,49] and involvement of transient receptor potential cation channel subfamily V member 1 (TRPV1).[62] Nevertheless, the evidence is currently insufficient to conclude that TRPV1 and purinergic receptors (subtypes 3–7) play a role in the etiology of UUI.

Finally, combining the results of gene expression and protein expression studies, we were also able to identify similarities of association in protein expression from the following pathways indicated by the transcriptome study of Cartwright et al.[23]: calcium-dependent signaling,[25,29] smooth muscle contraction,[19,29,46] G-protein coupled,[16,19,29] and cholinergic signaling.[19,29]

4.2 | Inflammation

Inflammatory responses and UUI appeared to be associated: serum CRP levels were significantly elevated in UUI patients in all three studies included,[31,33,45] and also levels of other inflammatory markers (interleukins, tumor necrosis factor-alpha, and MCP-1)[27,32,45,47] were elevated. The finding that urinary CRP was not elevated in UUI patients[31] implies that the elevated serum CRP levels do not originate from the bladder epithelium, but are possibly a reflection of submucosal inflammatory responses. How this relates to (the development of) UUI is unknown. Possibly, the different urinary microbiome of UUI patients may play a role.[63] Clearly, these aspects deserve attention to understand their role in UUI.

4.3 | Considerations and limitations

Despite the high number of studies retrieved by our search, only 37 studies were included. Due to an extensive search and the strict inclusion criteria, with the intention to restrict ourselves to a rather homogeneous and clear study population, many articles were excluded. We believe this method of reviewing associations in a well-defined population prevented the introduction of significant bias, which would make it difficult to link associations to specific symptoms, a problem Siddiqui et al.[9] described in their review of LUTS. However, despite this precaution, the populations (UUI vs. control) included in the various studies differed substantially, for instance in reporting of age, gender/sex, use of medications, BMI, and controls with different diseases, such as bladder cancer or a combination of these aspects. Therefore, some of the reported outcomes may still not be UUI-related, since these parameters could affect expression differences or influence genetic variant association. We decided to present these studies but marked them as (medium) high risk of bias.

With the use of different risk of bias tools for animal and human studies, we were able to assess study-specific determinants in an effort to reduce over/underestimation of the results. Generally, the risk of bias assessment showed a relatively high or unclear risk of bias which is a risk factor for an overestimation of reported associations. The unclear risk of bias was mainly due to the general lack of reporting standards and transparency in animal studies.

Most of the analyzed genes/proteins(-related products) were studied in isolation and involved individual studies, emphasizing the fragmented nature of the UUI research. Moreover, relatively low numbers of subjects were analyzed, possibly because of ethical considerations. This was also true for the gene knockout animal models. Nevertheless, these are very informative to demonstrate a direct cause-effect relationship between a certain gene and the UUI phenotype. The animal studies allowed researchers to study individual animals before they acquired UUI, something that is not possible in patients. Thus, the underlying molecular drivers can be studied in more detail. The value of these observations and the relation with clinical UUI remains to be firmly established, due to possible biological differences between animals and humans. The contribution of individual genetic variants to the etiology may also be limited. This may explain the lack of replicated results from the three GWASs.[15,17,18] In addition, these studies may have been underpowered. For a multifactorial symptom such as UUI, mostly occurring later in life, it is expected that the effect sizes of the variants will be low and therefore, a large group of participants is needed to find a statistically significant and replicable association.

5 | CONCLUSIONS

Signal transduction pathways and inflammation emerged as important biological processes potentially associated with UUI. A meta-analysis suggests a relation between an increased urinary level of NGF and UUI. This suggests aberrant signaling in both smooth muscle and nerve cells with the involvement of inflammation. Studies combining this information might lead to better insights in the

development and occurrence of UUI. Despite the high prevalence of UUI, only 37 studies met our inclusion criteria, implying the need for more focused and less fragmented future research with clearly defined populations. This systematic review provides an overview of genetic variants, gene, and protein expression changes in relation to UUI and therefore helps to formulate the actual knowledge gaps and research questions that need to be solved.

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SUPPORTING INFORMATION

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