Drake, M. J., Morris, N., Apostolidis, A., Rahnama'i, M. S., & Marchesi, J. R. (2017). The urinary microbiome and its contribution to lower urinary tract symptoms; ICI-RS 2015. *Neurourology and Urodynamics*, *36*(4), 850-853. https://doi.org/10.1002/nau.23006

The urinary microbiome and its contribution to lower urinary tract symptoms; ICI-RS 2015

Marcus J. Drake Nicola Morris Apostolos Apostolidis Mohammad S. Rahnama'i Julian R. Marchesi

Abstract

AIMS The microbiome is the term used for the symbiotic microbial colonisation of healthy organs. Studies have found bacterial identifiers within voided urine which is apparently sterile on conventional laboratory culture, and accordingly there may be health and disease implications

METHODS The International Consultation on Incontinence Research Society (ICI-RS) established a literature review and expert consensus discussion focussed on the increasing awareness of the urinary microbiome, and potential research priorities.

RESULTS The consensus considered the discrepancy between findings of conventional clinical microbiology methods, which generally rely on culture parameters predisposed towards certain "expected" organisms. Discrepancy between selective culture and RNA sequencing to study species-specific 16S ribosomal RNA is increasingly clear, and highlights the possibility that protective or harmful bacteria may be overlooked where microbiological methods are selective. There are now strong signals of the existence of a 'core' urinary microbiome for the human urinary tract, particularly emerging with ageing. The consensus reviewed the potential relationship between a patient's microbiome and lower urinary tract dysfunction, whether low-count bacteriuria may be clinically significant and mechanisms which could associate micro-organisms with lower urinary tract symptoms.

CONCLUSIONS Key research priorities identified include the need to establish the scope of microbiome across the range of normality and clinical presentations, and gain consensus on testing protocols. Proteomics to study enzymatic and other functions may be necessary, since different bacteria may have overlapping phenotype. Longitudinal studies into risk factors for exposure, cumulative risk, and emergence of disease need to undertaken.

Key words

Microbiome, microbiota, lower urinary tract symptoms, overactive bladder, urinary tract infection; LUTS; bladder pain syndrome

Introduction

“Microbiota” refers to the assemblage of microorganisms present in a defined environment. This is a crucial part of the “microbiome”, which can be used to refer to the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eurkaryotes, and viruses), their genomes, and the surrounding environmental conditions (1). The concept of a microbiota contrasts with infection, where pathogenic bacteria trigger an inflammatory immune response. The microbiome of the gut has been well documented (2), but less is known about the urinary microbiome. It is understood that for health, maintenance of the normal microbiome is essential (3) and this not only impacts on the risk of infection by pathogenic organisms, but is also likely to be implicated in the occurrence of immune mediated disease and carcinogenesis (4). Additionally the urinary or bladder microbiome may contain microbially encoded functions, which when expressed may be beneficial or detrimental to the host.

Sterility of healthy urine is thought to be maintained by host factors, of which the most important are;

1. Anatomical: the physical barriers between the source of pathogenic bacteria and the urinary tract
2. Urodynamic: ongoing flow of urine through the urinary tract, and complete bladder emptying when voiding
3. Anti-microbial: antibodies, proteins and factors that kill or restrict the ability of the microbes to infect

Urinary Tract Infection (UTI) is a common clinical problem, in which organisms are able to establish infection by expression of pathogenic features and/or impairment of at least one of the host defences. Conventionally, urine is considered to be sterile,

illustrated by the frequent absence of bacterial growth when urine is cultured from asymptomatic individuals and in the diagnostic setting. However, key studies using new techniques have found bacterial identifiers within voided urine which is apparently sterile when subjected to conventional laboratory culture, taken from patients with no associated symptoms of infection (5, 6).

The International Consultation on Incontinence Research Society (ICI-RS), meeting in Bristol in 2015, considered the emerging awarness of the urinary microbiome, discussing what potential links lie between urinary microbiota and urinary tract pathology, and identifying high priority research questions.

Is urine sterile?

Conventional microbiological methods using culture do not capture the full spectrum of urine bacterial species; growth conditions and culture media significantly affect bacterial yield, and organisms unable to grow in standard culture conditions will not be detected, e.g. anaerobic organisms. Potentially, the presumed sterility of urine may actually reflect the insensitivity of current routine culture methods. When non-culture based methods were used to study the urinary microbiota in mid stream urine (MSU) samples from 16 asymptomatic healthy individuals (26-90 years old), a very different picture emerges from that seen with culture on conventional media. 454 pyrosequencing of the 16S rRNA gene in conjunction with quantitative PCR (qPCR) of the 16S rRNA gene in urine samples allows measures of diversity and enumeration of the bacterial loads of each sample. The total number of genera identified from each person with respect to age was highly variable, and a total of 94 genera were obtained (6). Of these, over two thirds would not be routinely cultivated or not reported individually by standard microbiological investigations. Females had a more heterogeneous mix of bacterial genera than males and more typically had

representatives of the phyla *Actinobacteria* and *Bacteroidetes.* Both genders were mainly dominated by the phylum *Firmicutes.* Some age specific genera were also identified, i.e. *Jonquetella, Pavimonas, Proteiniphilum* and *Saccharofermentans.* Other separate studies have independently reported urinary bacteria using 16S rRNA gene sequencing (7, 8).

This preliminary data indicates that the human urinary tract is not ‘sterile’, but possesses a ‘core’ urinary microbiome, and that about two thirds of these bacteria would not be seen with conventional culture methods. The finding of a wide range of bacterial RNA is of interest, but this does not mean the presence of viable organisms. Instead the RNA identified could mean previous exposure, with long-lasting persistence of breakdown products, but not necessarily ongoing bacterial influence. Nonetheless, the reporting of the ability to culture bacteria using enhanced microbiological methods which are less selective for anticipated bacteria (9) suggests that some of these bacteria may be viable. Further research in this area is essential to understand better the role of a healthy urinary microbiome for bladder and urinary tract health, and how alterations to this with ageing might affect the balance between health and disease.

Does a patient’s Microbiome predict or reflect lower urinary tract dysfunction?

Some studies support an epidemiological association between the human urine microbiome and lower urinary tract symptoms (LUTS) or incontinence. The presence of significant bacteriuria in women strongly correlates with all storage symptoms (nocturia OR 3.56, p=0.02, urgency OR 6.66, p=0.01, urgency incontinence OR 2.92, p=0.046, nocturnal enuresis OR 4.21, p=0.01) and bladder pain (OR 2.82, p=0.049). Bacteriuria was significantly associated with all aforementioned symptoms apart from urgency incontinence even after adjustment for age, parity, symptomatic prolapse,

menopausal status and history of mid-urethral sling surgery (10). Amongst women with LUTS who had no acute frequency and dysuria, one out of five was found to have a positive MSU culture and one out of four sterile pyuria (11). Most of these women had overactive bladder syndrome (OAB) symptoms (74%). On the more obvious side, women who develop UTIs suffer significantly higher urine loss compared to those who do not experience UTIs (mean rate of urine loss x4.6/month as opposed to x2.6/month, p=0.04, in postmenopausal women). Further, among those who developed a UTI, the rate of urine loss was x1.5 higher during the first three days of a clinical UTI (12).

Distinguishable microbiota have now been identified between pure urgency urinary incontinence (UUI) or predominantly UUI patients and those with predominantly or entirely stress urinary incontinence (SUI). The latter two groups, however, appear to have significantly less diverse microbiota than the former two. In addition, the presence of bacterial DNA in the urine is associated with increased frequency of UUI episodes (13).

Controlled data suggest a greater number of bacterial species in women with UUI compared to non-UUI women, but also differences in the diversity of the bacterial species (14). The use of novel laboratory techniques, such as 16S rRNA gene sequencing and an exhanced culture protocol (expanded qualitative urine culture – EQUC), has allowed the identification of bacterial species which may not be detectable with the currently used microbiology analysis techniques (9). Accordingly, it has been found that non-UUI women may have more lactobacilli species than UUI women, while *Aerococcus* and *Gardnerella* species are more prevalent in UUI patients (14). Futher to higher mean daily number of UUI episodes, women who were

sequence-positive for urine bacteria were younger (55.8 vs 61.3 years old; p=0.0007) and had a higher body mass index (33.7 vs 30.1 kg/m^2; p=0.0009).

Two recent studies have reported observations on the associations between urine microbiota and response to treatment of UUI. Responders to solifenacin treatment were more likely to have fewer bacteria and a less diverse bacterial community at baseline than non-responders (15). Sequence-positive women responded better to Botulinum neurotoxin-A treatment and were less likely to experience a UTI post-instrumentation (16). However, more information is needed to interpret the relationship between the patient's microbiome and likely response to treatment of lower urinary tract dysfunction.

Is low-count bacteriuria clinically significant after all?

The term "bacteriuria" signifies the presence of bacteria in the urine, but where there are comparatively low bacterial numbers on culture, their presence may be disregarded as non-significant clinically. Nontheless, a prospective, cross-sectional study of prevalence of bacteriuria ≥10^3 CFU/ml on catheter specimens showed that incontinent women were four times more likely to have bacteriuria compared to continent controls, with 2 out of 3 bacteriuric specimens growing 'low-count' bacteriuria. The presence of bacteriuria was strongly associated with urodynamic increased bladder sensation (17). Further, following the use of enhanced culture techniques which could identify bacteriuria ≥10^2 CFU/ml, polymicrobial cultures were grown in 69% of chronic LUTS patients which were previously not considered with the standard MSU technique (18). The study of shed urothelial cells also allowed identification of several uropathogens, distinct between patients with chronic LUTS and controls (19). In light of such findings, considerations were raised about the

clinical value of currently used MSU culture techniques, while the implications for further research into the clinical significance of low-count bacteriuria are obvious.

Mechanisms associating micro-organisms with LUTS/OAB

Studies during acute UTIs have demonstrated invasion by *Escherichia coli* into the cytoplasm of urothelial cells both in animals and humans, with persistence of long-term intracellular bacterial reservoirs (20). Similarly, controlled studies of patients suffering from chronic LUTS showed that *Enterococcus faecalis* could subvert and invade the host urothelium; 75% of specimens from chronic LUTS patients had evidence of urothelial cell colonization, as opposed to 17% of the control samples (21). Increased apoptosis of urothelial cells noted in patients with increased pyuria is a possible mechanism for increased exposure of the underlying mucosal layer to irritative actions and, in consequence, to the generation of LUTS. Chronic inflammatory findings in a large proportion of OAB bladder specimens with inflammatory cells present in the lamina propria in 98.4% of cases could be a pointer to such a mechanism (22).

Another mechanism may involve host antimicrobial peptides (AMPs) which are essential components of normal host innate immune responses against infection and pathogen-induced inflammation. Studies of urine specimens from female pelvic floor surgery participants demonstrated significant correlations between UTI risk and both specific urinary microbiota and beta-defensin AMP levels. In addition, urinary AMP hydrophobicity and protease activity were greater in participants who developed UTI, and correlated positively with both UTI risk and symptoms (23).

Inflammatory markers are sometimes increased in patients with OAB (24), but no clear link with infection has been established. Nonetheless, in both UTI and OAB, the

prostaglandin (PG) system has been studied as a possible marker or modulating system. Increased urinary levels of Prostaglandin E_2, (PGE_2) and cyclo-oxygenase (COX-2) expression correlate with UTI and inflammatory processes, such as those induced by Bacillus Calmette Guerin (BCG) (25). Patients in whom UTI was treated with antibiotics have reduced urinary PGE_2 compared with those who have active disease (25). The role of prostaglandins in bladder control and in OAB has been the focus of many studies. PG receptors EP1 and EP2 are present in the urothelium, the detrusor and intramural ganglia of the guinea pig urinary bladder (26-29). Moreover, *in vitro* animal studies have shown that PGE_2 has an effect on non-voiding contractions of the urinary bladder (30).

Clinical studies have shown urinary PGE_2 levels to be significantly higher in OAB patients (31, 32). Furthermore, in patients with detrusor underactivity, the urinary level of PGE_2 was decreased when compared to that of patients without detrusor underactivity (32). In addition, the urine PGE_2 levels negatively correlate with maximum bladder capacity in OAB patients (32). The serum PGE_2 level was also shown to be increased in patients with OAB (33), with a significant decrease after intravesical botulinum neurotoxin-A treatment (33). Moreover, there has been the suggestion that patients with urgency urinary incontinence have higher serum PGE_2 versus those with continent OAB (34). Hence, PG-targeted therapy has been suggested for treatment of a variety of bladder dysfunctions (33). Clinical studies have shown that non-selective COX inhibitors flurbiprofen and indomethacin were associated with urodynamic and clinical benefits in a controlled study of 62 patients with detrusor overactivity (DO) (35, 36). Unfortunately, adverse effects such as nausea, vomiting, headache and gastrointestinal symptoms have prevented their use in the treatment of OAB (35, 36). A double-blind, placebo-controlled study of the non-

selective cyclooxygenase inhibitor ketoprofen (4 weeks intravesical treatment course) in 30 women with urodynamically proven DO showed complete relief of symptoms in 18 patients, without adverse effects (37).

PG-targeted therapy has also been explored for the treatment of underactive detrusor syndromes (38, 39). In combination with the cholinergic agonist bethanechol chloride, intravesical instillation with PGE_2 had limited therapeutic benefit compared to placebo (40). Other studies showed that intravesical instillation of PGE_2 reduced the time to restoration of detrusor function after gynecological surgery (41, 42).

To add another complicating factor, epigenetic causes of OAB need to be considered. Epigenetics is the study of heritable changes in gene expression that are not caused by alterations in DNA sequence. Currently, there are virtually no studies on epigenetic changes and OAB. The infection of mammalian tissues with bacteria, viruses and other pathogens results in the modification of the host cell epigenome, particularly DNA methylation. *In vitro* infection of bladder urothelial cells with uropathogenic *E. coli* results in hypermethylation of the tumor suppressor gene CDKN2A, providing proof-of-concept that uropathogenic infection modulates the host cell epigenome (43).

Future studies are needed to validate reported increased urinary PG levels in OAB, which might make them a useable biomarker for OAB. In addition, further studies are needed to examine the value of agents influencing the PG system, such as COX inhibitors and EP receptor modulators, as a possible treatment of OAB. The intravesical route of drug administration would be an interesting subject of study to bypass the gastrointestinal side effects of these drugs.

Research challenges

New technologies for isolating and sequencing DNA are emerging, which are enhancing experimental opportunities and bringing down costs. Thus, there are considerable opportunities for developing the field, and rapid progress may now become possible. The ICI-RS meeting identified that it is very desirable to establish the scope of microbiome across the range of normality and clinical presentations, with epidemiological studies to identify whether any individual bacteria or synergistic associations are significant. The challenge is considerable and would need extensive resources, so there is a need for additional pilot research studies to support proof-of-principle. Key to this is the importance of consensus on protocols, including the method of sample capture; voided urine is a convenient sample, but contains contributions from the entire urinary tract and perineum, which makes interpretation more complex and potentially open to question. Alternatives may require internal access, but this is likely to be poorly accepted by patients, and difficult to employ in general healthcare.

Little is known about what functions the microbiomic organisms provide in this niche. For example, is there a protective role for certain organisms equivalent to the vaginal *Lactobacillus*?

Since there is functional overlap in protein expression between different bacterial genera, the need for proteomics to study enzymatic and other functions may be necessary. Such expression may be relevant where considering potential clinical concerns, such as whether bacteria are able to re-activate host conjugated toxins or carcinogens by enzymatic processes (or conversely whether they produce anti-bacterial molecules which may afford protection against UTI pathogens, such as bacteriocins).

Bacterial-host interactions are potentially crucial. Does the host genotype or phenotype affect which bacteria can have importance for disease generation or maintenance? Does alteration of microbiome have epigenetic effects? This will be a complex but fundamentally interesting challenge. Bacterial induction of secondary responses is also challenging but may be more achievable. For example, does altered serum or urinary PG levels reflect a secondary or mechanistic component in microbiomic generation of functional LUT disorders?

Of course, a major interest lies in identifying whether there are there harmful organisms that can establish or maintain lower urinary tract disease. This may be in the form of a single organism, or a harmful combination. If such influence is identified, it raises the possibility to cure or prevent disease. Of course, it would be invaluable to know what mechanistic processes may drive the relevance- mediators, inflammation, or structural effects?

Finally, the risk factors for exposure, insights into lifelong cumulative exposure risk, and the importance of key events (e.g. catheterisation, immunosuppression, menopause) have to be understood.

Conclusions

Modern techniques avoiding selective culture indicate the presumption of sterility of urine is potentially wrong. There are many potential consequences of microbial influence which may be important for generation of symptoms and disease, or conveivably may be protective. To establish the relationship will require considerable research progress, but the findings potentially could be rewarding for understanding disease mechanisms and possibilities for clinical intervention.

Acknowledgements

None

Funding

None

References

1. Marchesi, J. R., and Ravel, J. (2015) The vocabulary of microbiome research: a proposal. *Microbiome* **3**, 31 doi: 10.1186/s40168-40015-40094-40165
2. Marchesi, J., and Shanahan, F. (2007) The normal intestinal microbiota. *Curr Opin Infect Dis* **20**, 508-513
3. Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D., Hirschfield, G. M., Hold, G., Quraishi, M. N., Kinross, J., Smidt, H., Tuohy, K. M., Thomas, L. V., Zoetendal, E. G., and Hart, A. (2015) The gut microbiota and host health: a new clinical frontier. *Gut*
4. Goulet, O. (2015) Potential role of the intestinal microbiota in programming health and disease. *Nutr Rev* **73 Suppl 1**, 32-40
5. Brubaker, L., and Wolfe, A. J. (2015) The new world of the urinary microbiota in women. *Am J Obstet Gynecol* **213**, 644-649
6. Lewis, D. A., Brown, R., Williams, J., White, P., Jacobson, S. K., Marchesi, J. R., and Drake, M. J. (2013) The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol* **3**, 41
7. Fouts, D. E., Pieper, R., Szpakowski, S., Pohl, H., Knoblach, S., Suh, M. J., Huang, S. T., Ljungberg, I., Sprague, B. M., Lucas, S. K., Torralba, M., Nelson, K. E., and Groah, S. L. (2012) Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med* **10**, 174
8. Siddiqui, H., Nederbragt, A. J., Lagesen, K., Jeansson, S. L., and Jakobsen, K. S. (2011) Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiol* **11**, 244
9. Hilt, E. E., McKinley, K., Pearce, M. M., Rosenfeld, A. B., Zilliox, M. J., Mueller, E. R., Brubaker, L., Gai, X., Wolfe, A. J., and Schreckenberger, P. C. (2014) Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* **52**, 871-876
10. Sorrentino, F., Cartwright, R., Digesu, G. A., Tolton, L., Franklin, L., Singh, A., Greco, P., and Khullar, V. (2015) Associations between individual lower urinary tract symptoms and bacteriuria in random urine samples in women. *Neurourol Urodyn* **34**, 429-433
11. Khasriya, R., Khan, S., Lunawat, R., Bishara, S., Bignall, J., Malone-Lee, M., Ishii, H., O'Connor, D., Kelsey, M., and Malone-Lee, J. (2010) The inadequacy of urinary dipstick and microscopy as surrogate markers of urinary tract infection in urological outpatients with lower urinary tract symptoms without acute frequency and dysuria. *J Urol* **183**, 1843-1847
12. Moore, E. E., Jackson, S. L., Boyko, E. J., Scholes, D., and Fihn, S. D. (2008) Urinary incontinence and urinary tract infection: temporal relationships in postmenopausal women. *Obstet Gynecol* **111**, 317-323
13. Brubaker, L., Nager, C. W., Richter, H. E., Visco, A., Nygaard, I., Barber, M. D., Schaffer, J., Meikle, S., Wallace, D., Shibata, N., and Wolfe, A. J. (2014) Urinary bacteria in adult women with urgency urinary incontinence. *Int Urogynecol J* **25**, 1179-1184

14. Pearce, M. M., Hilt, E. E., Rosenfeld, A. B., Zilliox, M. J., Thomas-White, K., Fok, C., Kliethermes, S., Schreckenberger, P. C., Brubaker, L., Gai, X., and Wolfe, A. J. (2014) The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *MBio* **5**, e01283-01214
15. Thomas-White, K. J., Hilt, E. E., Fok, C., Pearce, M. M., Mueller, E. R., Kliethermes, S., Jacobs, K., Zilliox, M. J., Brincat, C., Price, T. K., Kuffel, G., Schreckenberger, P., Gai, X., Brubaker, L., and Wolfe, A. J. (2015) Incontinence medication response relates to the female urinary microbiota. *Int Urogynecol J*
16. Pearce, M. M., Zilliox, M. J., Rosenfeld, A. B., Thomas-White, K. J., Richter, H. E., Nager, C. W., Visco, A. G., Nygaard, I. E., Barber, M. D., Schaffer, J., Moalli, P., Sung, V. W., Smith, A. L., Rogers, R., Nolen, T. L., Wallace, D., Meikle, S. F., Gai, X., Wolfe, A. J., Brubaker, L., and Pelvic Floor Disorders, N. (2015) The female urinary microbiome in urgency urinary incontinence. *Am J Obstet Gynecol* **213**, 347 e341-311
17. Walsh, C. A., Siddins, A., Parkin, K., Mukerjee, C., and Moore, K. H. (2011) Prevalence of "low-count" bacteriuria in female urinary incontinence versus continent female controls: a cross-sectional study. *Int Urogynecol J* **22**, 1267-1272
18. Sathiananthamoorthy, S., Gill, K., Swamy, S., Kupelian, A., Collins, L., and Malone-Lee, J. (2013) A MICROBIOLOGICAL ASSESSMENT OF THE ROUTINE MIDSTREAM URINE CULTURE TO IDENTIFY URINARY TRACT INFECTION IN PATIENTS WITH LOWER URINARY TRACT SYMPTOMS. *Neurourol Urodyn* **31**, 736-737
19. Khasriya, R., Sathiananthamoorthy, S., Ismail, S., Kelsey, M., Wilson, M., Rohn, J. L., and Malone-Lee, J. (2013) Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. *J Clin Microbiol* **51**, 2054-2062
20. Hunstad, D. A., and Justice, S. S. (2010) Intracellular lifestyles and immune evasion strategies of uropathogenic Escherichia coli. *Annu Rev Microbiol* **64**, 203-221
21. Horsley, H., Malone-Lee, J., Holland, D., Tuz, M., Hibbert, A., Kelsey, M., Kupelian, A., and Rohn, J. L. (2013) Enterococcus faecalis subverts and invades the host urothelium in patients with chronic urinary tract infection. *PLoS One* **8**, e83637
22. Apostolidis, A., Jacques, T. S., Freeman, A., Kalsi, V., Popat, R., Gonzales, G., Datta, S. N., Ghazi-Noori, S., Elneil, S., Dasgupta, P., and Fowler, C. J. (2008) Histological Changes in the Urothelium and Suburothelium of Human Overactive Bladder following Intradetrusor Injections of Botulinum Neurotoxin Type A for the Treatment of Neurogenic or Idiopathic Detrusor Overactivity. *Eur Urol* **53**, 1245-1253
23. Nienhouse, V., Gao, X., Dong, Q., Nelson, D. E., Toh, E., McKinley, K., Schreckenberger, P., Shibata, N., Fok, C. S., Mueller, E. R., Brubaker, L., Wolfe, A. J., and Radek, K. A. (2014) Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. *PLoS One* **9**, e114185
24. Ghoniem, G., Faruqui, N., Elmissiry, M., Mahdy, A., Abdelwahab, H., Oommen, M., and Abdel-Mageed, A. B. (2011) Differential profile analysis of urinary cytokines in patients with overactive bladder. *Int Urogynecol J* **22**, 953-961
25. Wheeler, M. A., Hausladen, D. A., Yoon, J. H., and Weiss, R. M. (2002) Prostaglandin E2 production and cyclooxygenase-2 induction in human urinary tract infections and bladder cancer. *J Urol* **168**, 1568-1573
26. Rahnama'i, M. S., Biallosterski, B. T., de Wachter, S. G., Van Kerrebroeck, P. E., and van Koeveringe, G. A. (2012) The distribution of the prostaglandin E receptor type 2 (EP2) in the detrusor of the guinea pig. *Prostaglandins Other Lipid Mediat* **99**, 107-115
27. Rahnama'i, M. S., de Wachter, S. G., van Koeveringe, G. A., van Kerrebroeck, P. E., de Vente, J., and Gillespie, J. I. (2011) The relationship between prostaglandin E receptor 1 and cyclooxygenase I expression in guinea pig bladder interstitial cells: proposition of a signal propagation system. *J Urol* **185**, 315-322

28. Rahnama'i, M. S., Hohnen, R., van Kerrebroeck, P. E., and van Koeveringe, G. A. (2015) Evidence for prostaglandin E2 receptor expression in the intramural ganglia of the guinea pig urinary bladder. *J Chem Neuroanat* **64-65**, 43-47
29. Rahnama'i, M. S., van Koeveringe, G. A., Essers, P. B., de Wachter, S. G., de Vente, J., van Kerrebroeck, P. E., and Gillespie, J. I. (2010) Prostaglandin receptor EP1 and EP2 site in guinea pig bladder urothelium and lamina propria. *The Journal of urology* **183**, 1241-1247
30. Rahnama'i, M. S., van Koeveringe, G. A., van Kerrebroeck, P. E., and de Wachter, S. G. (2013) The effect of indomethacin on the muscarinic induced contractions in the isolated normal guinea pig urinary bladder. *BMC Urol* **13**, 8
31. Cho, K. J., Kim, H. S., Koh, J. S., and Kim, J. C. (2013) Changes in urinary nerve growth factor and prostaglandin E2 in women with overactive bladder after anticholinergics. *Int Urogynecol J* **24**, 325-330
32. Kim, J. C., Park, E. Y., Hong, S. H., Seo, S. I., Park, Y. H., and Hwang, T. K. (2005) Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. *Int J Urol* **12**, 875-880
33. Rahnama'i, M. S., Uckert, S., Hohnen, R., and van Koeveringe, G. A. (2013) The role of phosphodiesterases in bladder pathophysiology. *Nature reviews. Urology* **10**, 414-424
34. Hegele, A., Knippschild, S., Frohme, C., Hanze, J., Olbert, P., and Hofmann, R. (2014) Changes in prostaglandin E2 in patients with idiopathic overactive bladder syndrome after botulinum toxin type A treatment: is there a clinical benefit? *BMC Urol* **14**, 85
35. Cardozo, L. D., and Stanton, S. L. (1980) A comparison between bromocriptine and indomethacin in the treatment of detrusor instability. *J Urol* **123**, 399-401
36. Cardozo, L. D., Stanton, S. L., Robinson, H., and Hole, D. (1980) Evaluation of flurbiprofen in detrusor instability. *Br Med J* **280**, 281-282
37. Sprem, M., Milicic, D., Oreskovic, S., Ljubojevic, N., and Kalafatic, D. (2000) Intravesically administered ketoprofen in treatment of detrusor instability: cross-over study. *Croat Med J* **41**, 423-427
38. Bergman, A., Mushkat, Y., Gordon, D., and David, M. P. (1992) Prostaglandin for enhancing bladder function after vaginal surgery. Does it work? *The Journal of reproductive medicine* **37**, 320-322
39. Delaere, K. P., Thomas, C. M., Moonen, W. A., and Debruyne, F. M. (1981) The value of intravesical prostaglandin E2 and F2 alpha in women with abnormalities of bladder emptying. *Br J Urol* **53**, 306-309
40. Hindley, R. G., Brierly, R. D., and Thomas, P. J. (2004) Prostaglandin E2 and bethanechol in combination for treating detrusor underactivity. *BJU Int* **93**, 89-92
41. Bergman, A., Mushket, Y., Gordon, D., and David, M. P. (1993) Prostaglandin prophylaxis and bladder function after vaginal hysterectomy: a prospective randomised study. *Br J Obstet Gynaecol* **100**, 69-72
42. Koonings, P. P., Bergman, A., and Ballard, C. A. (1990) Prostaglandins for enhancing detrusor function after surgery for stress incontinence in women. *J Reprod Med* **35**, 1-5
43. Tolg, C., and Bagli, D. J. (2012) Uropathogenic Escherichia coli infection: potential importance of epigenetics. *Epigenomics* **4**, 229-235