Understanding the Link Between Gut Microbiome and Urinary Stone Disease

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Abstract

Purpose of Review With recent advances in sequencing technologies and increasing research into the gut microbiome (GMB), studies have revealed associations between the GMB and urinary stone disease (USD). We sought to determine whether the evidence pointed towards a few specific gut bacteria or the broader GMB network is seemingly responsible for this relationship.
Recent Findings Initially, *Oxalobacter formigenes* (*OF*) was pursued as the main link between GMB and USD given its ability to degrade oxalate in the gut. However, the latest studies consistently suggest that the entire GMB is much more likely to be involved in handling oxalate absorption and other risk factors for urinary stone formation, rather than just a few microbiota.
Summary The GMB has complex networks that are likely involved in the pathophysiology of USD, although the causal mechanisms remain unclear. With increasing interest and research, potential modalities that act on the GMB may help to prevent incidence of USD.

Keywords Gut microbiome · Endourology · Urinary stone disease*Oxalobacter formigenes*

Introduction

Urinary stone disease (USD) is a growing epidemic in the USA that results in over $10 billion in healthcare costs annually and affects 8.8% of the US population [1]. The prevalence has increased by 70% over the past 30 years [1], yet the reason for the increase in USD incidence remains to be clarified.

Recent advances in gut microbiome (GMB) sequencing technology have enabled innovative breakthroughs that have revealed associations between the gut microbiome (GMB) and various health issues. Metabolic diseases like asthma [2], inflammatory bowel disease [3, 4], and cardiovascular disease [5] have all been linked to changes in the GMB.

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Although far removed from the organ systems it may affect, the idea of dysbiosis, an imbalance of the GMB, is being tied to the pathophysiology of many diseases and ongoing research continues to further understand these relationships. One early and innovative study that demonstrated the ability of the GMB to affect physiology showed that obesity could be passed on in a murine model through fecal transplants [6, 7]. Similarly, both obese and diabetic patients have been to found to have unique GMB profiles [8], and GMB transplants have been shown to impact insulin sensitivity [9]. That both obesity and DM, both known risk factors for urinary stone disease (USD) [10, 11], have a unique GMB, it has become plausible that indeed the pathophysiology of USD may to some degree be influenced by the bacteria that inhabit our intestines.

Before the recent technological advances that have spurned interest in microbial sequencing, researchers previously investigated the ability of the single species *Oxalobacter formigenes* (*OF*) to metabolize oxalate. Several studies suggested that a paucity of *OF* could be a risk factor for USD [12, 13]. Knowing that USD has been linked to metabolic comorbidities including asthma [11, 14], diabetes, and obesity and that GMB has been implicated in each of these diseases has ushered in a new era that considers the GMB as a whole unit that works together in a supportive network of microbes, that when disrupted by illness, diet or antibiotics can become part

of the pathophysiology of many disease states, USD included [15•, 16]. To test this hypothesis, our group recently compared the GMB profiles of stone formers and controls and discovered that stone formers had a unique GMB profile [17].

The purpose of this review is to survey the latest literature surrounding the effects of the GMB on USD. This review should introduce the reader into the current thinking about the GMB and how it may influence USD. The field of study examining GMB and USD is only in its outset and much remains to be discovered about this association.

Materials and Methods

We performed extensive literature searches in PubMed for kidney stones and gut microbiome/microbiota in PubMed (1946 to September 2018), Cochrane CENTRAL (1946–September 2018), and Web of Science Core Collection-Science (1985–September 2018).

Historical Perspective

The advent of microbial analysis started hundreds of years ago in the seventeenth century, when Antonie van Leeuwenhoek developed the first microscope strong enough to see bacterial organisms [18]. Since then, with the advent of high-throughput sequencing biotechnology has drastically advanced allowing researchers to promptly analyze specific gut microbiomes, which has opened a gateway into the field of GMB research. Given these advances, many researchers have unveiled connections between GMB and several diseases, such as cardiovascular disease [5], obesity [7], inflammatory bowel disease [3, 4], asthma [2], and now USD [17, 19•, 20•].

Approximately 80% of kidney stones are comprised of calcium and oxalate. Urinary oxalate is a mix of endogenous and dietary oxalate metabolism [21–23]. Both dietary and endogenously produced oxalate are excreted almost entirely in the urine. In the healthy state, oxalate homeostasis is thus a balance between intestinal secretion and absorption. One transporter in particular has been identified in the intestine with an affinity for oxalate. Slc26A6, present in the small intestine, the distal colon, and in the kidney, promotes oxalate secretion [24–27]. Because of the ability of *Oxalobacter formigenes (OF)* to metabolize oxalate, studies have evaluated the potential for this single species to lower urinary oxalate [12, 13]. In addition to metabolizing oxalate, *OF* may have a more complex interplay with bowel physiology by regulating the intestinal anion exchanger Slc26A6 [28, 29]. Thus *OF* is now considered to have two means by which it can lower urinary oxalate; (1) metabolize intestinal oxalate into formate and CO_2 which will be excreted in the stool thereby lowering available oxalate for intestinal absorption; (2) by stimulating the intestinal anion exchanger Slc26A6, *OF* can induce the secretion of oxalate from the plasma back into the intestine. Another example of the potential for *OF* to lower urinary oxalate was shown using a mouse model of primary hyperoxaluria (PH1) where colonization with *OF* was shown to decrease urinary oxalate 95% and plasma oxalate 50% [30]. Another study, culturing *OF* from the stool of stone formers and controls, found that patients colonized with *OF* were associated with a 70% decreased risk for being a recurrent stone former [31].

Nonetheless, enthusiasm for *OF* use in isolation has been dampened by other follow-up studies that show that experimental *OF* colonization appears temporary, may be dependent on a narrow concentration of intraluminal calcium, oxalate, and pH [30], and was recently found to be ineffective at lowering urinary oxalate compared to placebo in a phase III clinical trial [32••]. With the onset of recent interest in high-throughput sequencing of the GMB, researchers have begun unveiling the link between the GMB and USD. Aside from the specific mechanisms of *OF*, little is known about the role of the entire GMB complex in USD with a strong need for further development of knowledge underlying this association.

Unique Findings in the GMB of Urinary Stone Patients

Aware of the previous work on *OF* and prior associations between GMB and comorbidities associated with USD, we were motivated to perform a pilot study to identify differences in the GMB of human stone formers compared to matched controls [17]. Sequencing of the GMB of a total of 29 patients revealed 178 unique bacterial genera. Of these, *Bacteroides* and *Prevotella* were weighted the most significantly and comprised 42% of the case and 45% of the control abundance respectively. Notably, *Bacteroides* was more abundant in the stone former group and *Prevotella* was more abundant in the non-stone former group. A multivariate analysis revealed a statistically increased risk for *Bacteroides* (OR = 3.26, p = 0.033) and an inverse association with *Prevotella* (OR = 0.37, p = 0.043) for stone formers compared to controls.

A larger GMB study with 52 stone formers and 48 controls found similar abundance of *OF* amongst the stone formers and controls [20•]. Thus, they went on to perform shotgun metagenomics analysis of oxalate metabolism in five stone formers and five controls in order to identify those bacteria with genes that code for enzymes responsible for oxalate degradation. Two such enzymes, formyl-CoA transferase and oxalyl-CoA decarboxylase, were found to be less well represented in stone formers compared to controls. Theoretically, the decreased expression of oxalate decarboxylase could lead to more available oxalate for gut absorption and thus higher urinary oxalate, increasing the risk for stone formation. The

group also demonstrated that the relative abundance of these two genes inversely correlated with the urinary oxalate excretion. Urinary citrate inhibits the formation of urinary stone crystallization [33•], and thus, increased urinary citrate levels may serve as a potential method of preventing stone formation. Other follow-up studies have additionally revealed distinct GMB profiles amongst stone formers, showing differences in the alpha and beta diversities, statistical measures of differences in microbial networks [19•, 34]. A larger study with more human gut microbiome samples from stone formers and non-stone former controls is required to confirm the findings of this study.

A recent study using over 8000 GMB samples from the American Gut Project, an open source project aiming to analyze the GMB of thousands of participants, examined the networks of microbiota associated with OF [35]. Samples collected from the general public, not classified by stone formers or controls, OF was present in approximately 30% of samples and represented a very small < 0.001% of the bacterial abundance. Phylogenic diversity, a sign of GMB health, tended to increase with increased OF abundance [36]. Although stone information was not available in this dataset, this study nicely demonstrated that intricate networks of microbiota in the GMB have complicated interactions, which could functionally respond differently to stressors.

Fecal Transplants Can Affect Metabolism and Thereby Urine Chemistry

Fecal transplant, whereby ones GMB is transplanted into the host, can introduce useful changes in host gut microbiota. Recently, they have been paramount in the treatment of *Clostridium difficile* infections (CDI) and exhibit potential therapeutic benefits in treating inflammatory bowel disease, obesity, and metabolic syndrome [37–39]. With our knowledge of the differences in GMB amongst stone formers compared to controls, we set out to establish that in fact, the GMB does have the capacity to alter stone risk by altering urinary chemistry know to be important for stone formation. To do this, we transplanted fecal microbiota from healthy mice into germ-free mice. At 4 weeks following fecal transplants, the germ-free mice showed statistically significant decreases in urinary calcium, oxalate, and ammonium as well as statistical increases in urinary pH. Additionally, the intestinal alkali absorption was found to be increased to statistical significance [40••]. These data indicated the potential capability for fecal transplants to alter the urinary pH and chemistry in a manner beneficial to USD patients.

Data from our lab has indicated fecal transplant of the whole GMB from healthy animals into germ-free mice was also associated with changes in various intestinal transporters important in the urinary excretion of metabolites contributing to USD development. Others have shown that Slc26a6 receptor expression can be modified in the presence of certain bacteria [29]. Together, these findings suggest that in addition to OF, there is likely a larger role for the GMB in regulating intestinal absorption of electrolytes important for stone disease.

Another study transplanted the whole GMB from the oxalate-metabolizing wild mammalian herbivore, *Neotoma albigula*, to the laboratory rat, *Rattus norvegicus*, which are not capable of degrading oxalate [41]. They found that the transplanted laboratory rats showed increases in oxalate degradation that persisted at 9 months after transplant. This finding has important indications suggesting that GMB transplants, rather than OF transplants alone, can have significant impacts on the degradation of oxalate on a long-term basis in the mammalian gut. Furthermore, this study demonstrates that whole GMB may reduce the risk of recurrent stone disease.

Given the previous research into the role of the GMB and fecal transplants in treating IBD, obesity, and metabolic syndrome, it is feasible that fecal transplants may play a role in prevention and treatment of USD. The data from our lab combined with the other studies bring to light the novel concept that experimental changes in the fecal microbiome have the ability to alter urinary parameters and may be linked to the pathophysiology of USD. This direction of research may lead to promising therapeutic interventions to prevent stone formation.

Alterations of the GMB with Antibiotics

With the discovery of the association of the GMB and USD, oral probiotics were the first potential therapeutic modality for USD explored by many researchers. The goal was to identify if manipulation of the GMB using probiotic oral supplements could affect oxalate metabolism and prevent the formation of urinary stones. However, trials using probiotics containing *Oxalobacter*, *Lactobacillus*, and/or *Bifidobacterium* spp., have not shown promising results in reducing urinary oxalate [42]. These results suggest the therapeutic modalities for the prevention of USD need to focus on the more complex networks of the GMB beyond that of single bacterial species.

Antibiotics have profound effects on the GMB, causing rapid perturbations within days of antibiotic use with lasting effects for over 6 months [43, 44]. An initial study that examined subjects who were on antibiotics for treatment of *Helicobacter pylori* (HP) was then found to have reduced colonization with OF [44]. These antibiotic-induced changes in the GMB may have an impact on the development of USD through alterations in the GMB. Tasian et al. recently identified an association between USD and antibiotic use in the 3–12-month period prior to the stone event [45]. The study found that more recent exposure to antibiotics (within 3–6 months)

was associated with a higher odds of USD, and the authors postulated that perturbations in the urinary or gut microbiome could be associated with this increased risk of stone disease. Another study found that the use of antibiotics causes significant drops in the diversity and richness of the GMB, which has profound effects on the symbiosis of the GMB [46]. The reductions in OF and GMB diversity associated with antibiotic use may indeed play a significant role in the development of USD.

Urinary Microbiome

Although it was considered to be a sterile environment for many years, the urinary tract is now considered to have its own urinary microbiome (UMB), according to recent studies [47]. Initially, researchers questioned the pathogenesis of interstitial cystitis or painful bladder syndrome (IC/PBS), which was traditionally thought to have an unknown etiology [48]. However, these painful chronic conditions responded in certain cases to antibiotic therapies [49, 50]. This lead researchers to characterize the urinary microbiome, which did not grow bacterial cultures under traditional methods [51]. Sequencing of the urinary microbiome, however, revealed reduced diversity in the IC/PBS urine samples and also showed a higher abundance of *Lactobacillus* compared to controls [51]. Similar studies were performed for chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), revealing differences in clustering and diversity in men with CP/CPPS compared to age-matched controls [52]. Studies have revealed unique changes in the urinary tract microbiota in those with neurogenic bladder dysfunction [53], urgency urinary incontinence [54], and sexually transmitted diseases [55].

New information about the relationship between the urinary microbiome and the pathogenesis of USD has begun to surface in the past few years. High-throughput sequencing has shown that USD is associated with the Enterobacteriaceae genus in the urinary microbiome, which includes the *Escherichia coli* species [56]. The researchers then inoculated the urinary tracts of mice with uropathogenic *E. coli* to determine if the presence of this bacteria affected calcium deposition. Indeed, they found that the calcium deposition was 2.7-fold higher after inoculation with *E. coli* [56]. Another small study demonstrated that bacteria could be isolated from urinary stones, including *E. coli* and *Pseudomonas* [57]. A few theories explaining the association between urinary bacteria and USD are that the bacteria adhere to the crystals and promote agglomerations or by bacterial production of citrate lyase, which would decrease the levels of citrate in the urine and promote stone formation [57, 58]. The field of research into the role of the urinary microbiome on USD pathogenesis is still developing, with the need for research into the network of stone and urinary microbiomes.

Conclusions

The incidence of USD has steadily increased over the past few decades, despite efforts to address dietary risk factors for stone disease. Discoveries in the pathophysiology of USD and advances in sequencing technology have opened a new field of study into the role of the GMB in relation to USD. The various potential therapeutic modalities for the prevention of USD, with respect to the GMB, focus on probiotic and antibiotic interactions with the sensitive GMB. As mentioned above, the attempts at probiotics utilizing *Oxalobacter*, *Lactobacillus*, and/or *Bifidobacterium* spp. have not yielded promising results or persistent effects. It is reasonable that the metabolism of oxalate, calcium, and citrate is regulated in a complex manner that extends beyond the capacities of a few bacterial species. Although we are far from a therapeutic solution, it is plausible that therapeutic innovations that alter the GMB may play a role in preventing USD and suppressing the growing rates of incidence.

Given the probable likelihood of the entire GMB in regulating the metabolism of oxalate and other risk factors for USD, microbial transplants from mammals with oxalate-degrading bacteria could play a role in the search for treatments to prevent USD. Fecal transplants from healthy subjects without USD may have a larger impact on the degradation of oxalate and urinary excretion of oxalate. Nonetheless, larger, multi-institutional studies are likely required to collect more information about the networks of GMB within subjects with and without USD. Further lab research is also required to identify causal mechanisms that underlie this relationship. The role of the GMB in the pathogenesis of USD is in its early stages of development and, with continued research efforts, more will be understood about this interaction and about what interventions could be developed to modulate USD development.

Compliance with Ethical Standards

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