Investigating Associations Between the Prostate Microbiome and Prostate Size in BPH

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common condition that can cause bothersome urination symptoms such as weak stream, urgency, and nocturia as well as urinary retention and kidney damage. An estimated 70% of men between the ages of 60-69 are affected by BPH and incidence increases by age[1]. With an increasingly older population in the US, the prevalence of BPH is expected to rise – and with it, the already-high cost and burden on the healthcare system due to doctor's visits, medications, surgeries, and related complications[2]. Unfortunately, the etiology of BPH is not well understood at this time making prevention impossible. Recent research on the human urinary tract microbiome has sparked interest on its role in a variety of urologic diseases and conditions, especially those that may be mediated by an inflammatory pathway[3].

As chronic prostatic inflammation is implicated in the pathogenesis of BPH, it is possible that disruptions in the prostatic microbiome, or collection of resident microorganisms, facilitates the onset of BPH. Previous studies found evidence for a relationship between the severity of lower urinary tract symptoms (LUTS) and the degree of chronic prostatic inflammation[4]. More recently, the degree of LUTS has been associated with distinct microbiota of the upper and lower urinary tract[5]. However, to our knowledge the literature has not yet sampled the prostatic microbiota itself. In our preliminary data, prostate biopsy cores were obtained through either rectally accessed or perineally accessed routes to screen for prostate cancer. Samples were subjected to DNA isolation and high throughput 16S sequencing, along with bacterial isolations. Results indicated that while the prostate microbiome associated with contaminants from the route of access, through bioinformatically[6] removing contaminants, a distinct prostate microbiome was found that could not be attributed to either rectal or perineal contamination. Furthermore, diverse bacteria were isolated from the prostate tissue itself, which again could not be associated to contaminants. These data show that a distinct microbiome exists within the prostate and thus may influence physiological factors such as prostate size. In this study, we sought to determine associations between age-independent prostate size and microbiome.

METHODS

Men over 18 years old undergoing Holmium Laser Enucleation of the Prostate (HoLEP) for BPH with no history of prostate cancer, prostate surgery, or pelvic radiation were recruited. Patients were excluded if they had a positive preprocedural urine culture, recent UTI requiring antibiotics, bladder stones, or if they were catheter-dependent due to obstruction. From each patient, prostate tissue, midstream urine, and urethral and specimen container swabs were collected. All non-prostate samples were used as contamination controls. Patient data such as age, prostate-specific antigen (PSA) level, BPH symptoms, and prostate size were recorded. All samples underwent DNA extraction and 16S sequencing, followed by analysis in R statistical software with Dada2, Phyloseq, Decontam, and Vegan packages. After quality control, reads associated with the contamination controls and other negative controls were removed. High-quality, decontaminated data were assessed for diversity (alpha, beta, taxonomy). The correlation between amplicon sequence variants (ASVs) and patient metrics were quantified through Sparcc correlations, which was designed for count matrix correlations.

RESULTS

20 patients qualified, consented, and were analyzed in this study. Mean age was 68.6 years, mean PSA was 3.4 ng/mL, and mean prostate size was 107.9 g. From all samples, 4368 taxa were classified. After bioinformatic decontamination of samples with the negative controls, diversity analyses showed site-specific differences between the urine, urethral swab, and prostate microbiomes were greater than inter-individual variability, indicative of distinct microbiomes in each sample origin (Figure 1). After removal of host, contaminate, and urine/urethral reads, 983 taxa from the prostate remained. The prostate microbiome was dominated by the Proteobacteria phylum, which includes known uropathogens such as *Enterobacter*, *E. coli*, others, and unidentified taxa that were unresolved to the genus level (Figure 2).

Largely, clinical metadata did not associate with alpha and beta diversity, with the exception of alpha diversity vs. incontinence and urinary urgency (p=0.07 and 0.06, respectively), and beta diversity vs. nocturia (p=0.076). Alpha diversity compared to PSA and age similarly was not significant (p=0.48 and p=0.64, respectively). However, a slight negative correlation between alpha diversity and prostate size was observed (p=0.09) (Figure 3). When qualitatively comparing the microbiomes associated with patient characteristics, there was no overlap found between flora associated with age, and flora associated with prostate size or PSA, indicating that these microbiome associations are unique (Figure 4).

Common uropathogens were positively associated with prostate size. These included five ASVs belonging to *Enterobacter cloaceae* (p=0.02-0.03), four Planococcaceae ASVs (p=0.008-0.03), and four *Acinetobacter* (p=0.014-0.026). Only one ASV in the *Ralstonia* genus exhibited a significant association with age (p=0.015).

CONCLUSIONS

This study is the first to characterize the prostatic microbiome in BPH and to link prostate size to specific common bacterial uropathogens while controlling for age and contamination. We observed unique microbiomes originating from urine, urethral swabs, and prostate samples. Other similar published studies on BPH and the microbiome either used midstream urine or did not correct for possible sources of contamination[7]. As such, these findings are more robust than elsewhere described in the literature. Further research with a larger sample size and culturomics will provide insight into the mechanisms of how the prostate microbiome contributes to enlarged size, and may elucidate further associations between patient metadata and their respective prostatic microbiomes.

Figure 1: Phylogenetic diversity by sample origin

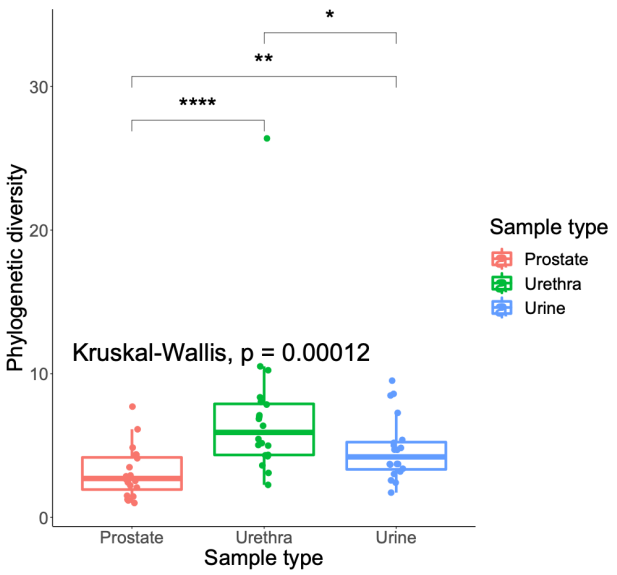


Figure 2: Prostatic microbiome taxonomy after urine and urethral decontamination

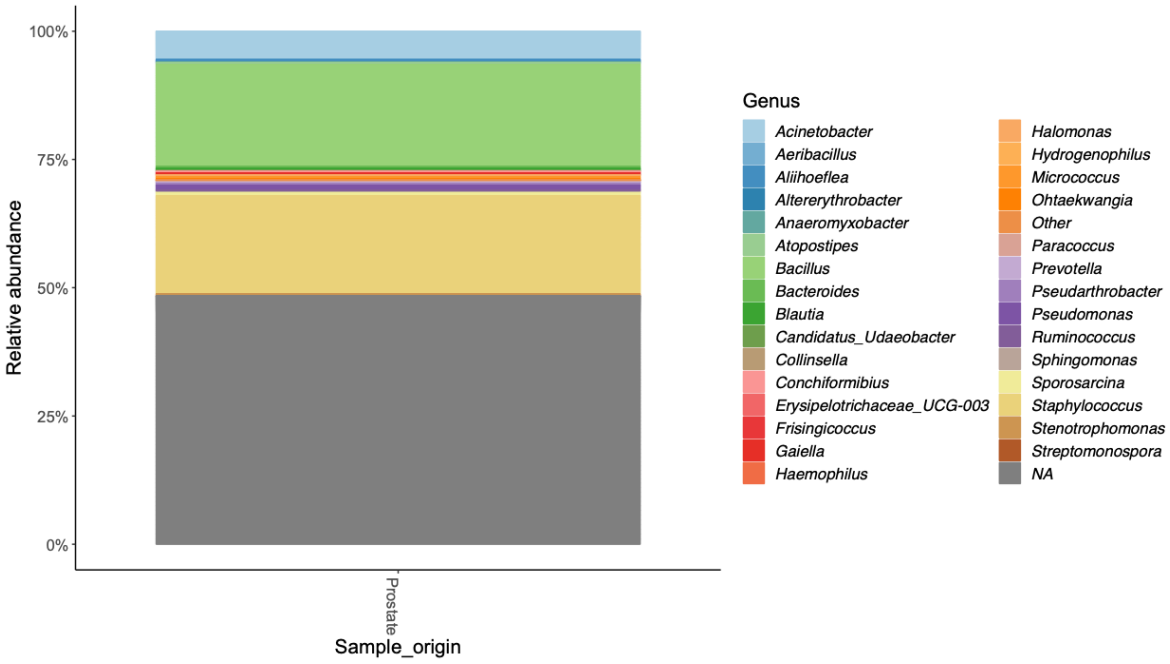


Figure 3: Alpha diversity of prostatic microbiome compared against age, prostate size, and PSA

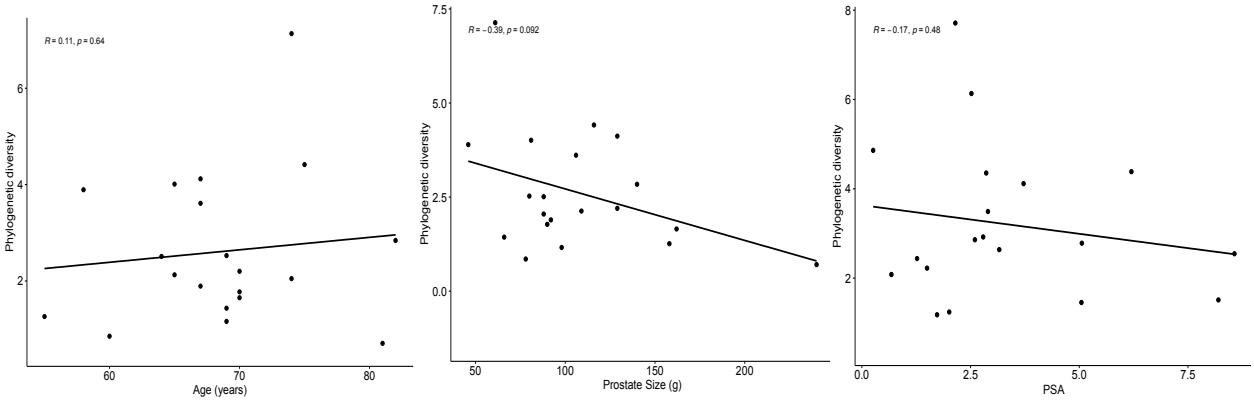
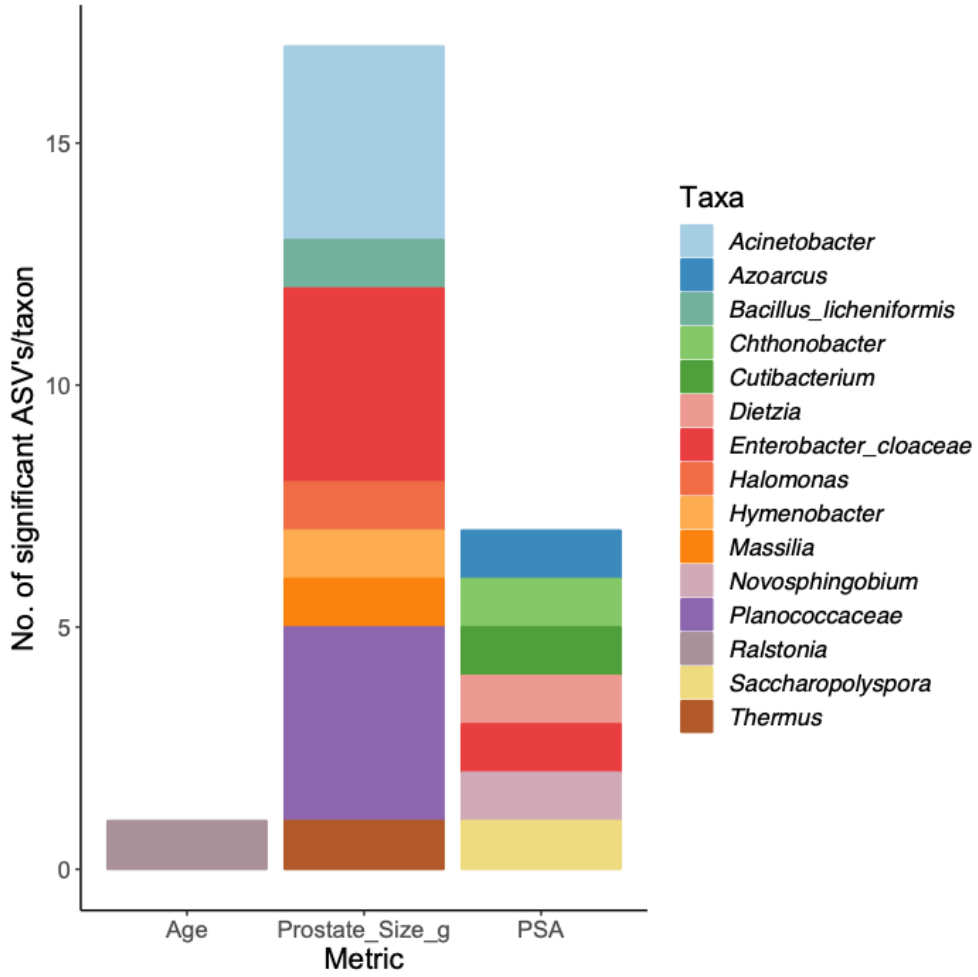


Figure 4: Taxa positively associated with age, prostate size, and PSA



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