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Identification of an intestinal microbiota enterotypes in ageing man diagnosed with benign prostatic hyperplasia (BPH)

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The intestinal microbiota, in terms of both composition and functioning, exerts a significant influence on the human body. Disturbed microbiota is a common occurrence in the context of numerous diseases. The available evidence increasingly points to a correlation between this condition and the development of prostate diseases, including benign prostatic hyperplasia and prostate cancer. 16 S sequencing of the V3-V4 region was performed and then evaluated alpha and beta diversity of the faeces microbiota of healthy (control group, *N* = 81) and BPH patients (study group, *N* = 76). The exploration of enterotypes involved the application of the Dirichlet-Multinomial model, executed for selecting community types. The study revealed no statistically significant difference in alpha diversity between the control group and the group of patients diagnosed with BPH. However, a significant difference was observed in beta diversity (Permanova test: F-value = 5.56, p-value < 0.001). The identification of enterotypes revealed significant differences between the healthy male cohort and those diagnosed with BPH ($p = 0.035$). In the cohort of men with BPH, the most prevalent was enterotype 3, characterized by a predominance of Blautia, Bacteroides, and Streptococcus. The occurrence of enterotype 3 was associated with an increased likelihood of BPH, exceeding threefold that of enterotype 1 (OR = 3.24). These findings suggest that alterations in the gut microbiota, particularly the presence of enterotype 3, may serve as a microbiological pattern associated with BPH.

Keywords Benign prostatic hyperplasia, Gastrointestinal Microbiome, Gut enterotypes

Benign prostatic hyperplasia (BPH) is a common, chronic and progressive urological disorder of ageing men. Histologically, BPH is characterised by proliferation of prostate stromal cells leading to enlargement of the gland in its transitional zone, which is clinically manifested as lower urinary tract symptoms (LUTS)[1].

The incidence of benign prostatic hyperplasia (BPH) is increasing at a rapid rate on a global scale. As evidenced by an inter-centre systematic analysis based on data collected from 2000 to 2019 in 204 countries, there were 94 million confirmed cases of BPH (in men aged ≥ 40 years) worldwide in 2019, representing a significant increase from the 51.1 million cases observed in 2000 [2]. Statistical data suggests that the prevalence of BPH will continue to increase in the near future due to the increasing life expectancy of men[2].

BPH significantly reduces men's quality of life (QoL) by affecting daily activities and psychological state[3]. Furthermore, BPH can result in a number of complications, including acute or chronic urinary retention, urinary tract infection, bladder stone formation, bladder wall damage, haematuria, kidney damage and erectile dysfunction[4]. It is therefore of great importance to take measures to minimise the risk of developing BPH.

The precise mechanism by which BPH develops remains unclear. It is established that the aetiology of BPH is multifactorial[1]. The principal risk factor is the age of the male subject. It is estimated that the prevalence of benign prostatic hyperplasia (BPH) in men is 50% in their sixth decade of life and 70% in their seventh decade. Furthermore, a longitudinal study revealed that the prostate increases in size with age by 2.5% (median growth

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rate) (0.6 ml) per year. Nevertheless, the rate of prostate growth exhibits considerable inter-individual variability[5]. Other risk factors for BPH include genetic predisposition and modifiable factors such as: metabolic syndrome and its components, obesity, dyslipidaemia, type 2 diabetes and arterial hypertension[6]. The pathogenesis of BPH is also associated with hormonal dysfunction - an increase in serum levels of estradiol and dihydrotestosterone (DHT) and DHT-dependent growth factors, and a predominance of cell proliferation over cell death[1,3]. In addition, chronic inflammation - caused by microbial, bacterial or viral factors, hormonal changes, MetS, bowel disease and an autoimmune response, contributes to the initiation of pathological changes in the prostate and thus to benign hyperplasia[7].

Recently, there has been increasing research into the composition and function of the gut microbiota and its impact on the development of prostate disease: BPH[8–14] and prostate cancer (PCa)[15–19], and the term 'gut-prostate axis' has been proposed. The precise mechanism by which the gut microbiome exerts its influence on the prostate remains unclear. It seems reasonable to posit that the dysbiosis of the gut affects the prostate indirectly by causing systemic chronic inflammation. It is possible that inflammatory mediators (cells and cytokines) from the gut may reach the gland via the systemic circulation, thereby causing localised inflammation and stimulating growth factors in the prostate stroma. This may, in turn, lead to the development of prostate disease[20,21].

The gut microbiota is a dynamic system that changes according to health status, medications taken, dietary habits and physical activity. Changes in the composition and function of the microbiota (referred to as "dysbiosis") can have secondary effects on health, including the creation of an inflammatory environment. Importantly, the composition of the gut microbiota varies between individuals and is individually specific. It is therefore useful for analysis to systematise bacteria into stable groups with similar characteristics - enterotypes.

The concept of enterotypes, which allows the grouping of gut bacteria into functional types, was introduced by Arumugam et al. (2011)[22]. Initially, three main enterotypes were distinguished: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). Within the *Ruminococcus* enterotype there are related groups of the order *Clostridales*, unclassified *Lachnospiraceae* and *Blautia*. However, in the course of research, depending on the microorganism grouping strategy, enterotypes specific to a particular disease or metabolic status of the organism have been identified[23–26], encompassing from two to four enterotypes[27]. Importantly, enterotypes remain unchanged under the influence of differentiating factors such as age, gender, geographical and cultural factors. In addition, long-term dietary habits influence the stability of specific enterotypes, which have different metabolisms and use different energy components, including carbohydrates, resistant starch, fibre, proteins and animal fats[28].

The principal objective of our investigation was to ascertain the profile and discrepancies in the composition of the gut microbiota. Our study represents the inaugural attempt to identify enterotypes in men with and without BPH.

Materials and methods

Patients

The study group included 76 men diagnosed with and treated for BPH who underwent transurethral resection of the prostate (TURP) at the Clinic of Urology and Urological Oncology, Pomeranian Medical University, Szczecin, Poland. The men were aged 49–79 years (mean age: 65.8 years, median: 67 years). The diagnosis was based on the results of the International Prostate Symptom Score (IPSS) questionnaire (with a question on overall quality of life - QoL), long-term symptoms, reduced flow rate (Qmax) or urinary retention, and increased prostate volume. BPH was confirmed in the prostate tissue removed during the TURP procedure.

The control group included healthy volunteers ($n = 81$) aged 45–72 years (mean: 54.7, median: 54) with a prostate size ≤ 30 ml and PSA less than 2.5 ng/ml. In this group, the IPSS questionnaire score did not exceed seven points, and the patients did not report any symptoms of BPH.

Exclusion criteria were: active cancer, alcoholism, thyroid disease, use of glucocorticosteroids and antibiotics in the six months prior to the examination. The study was approved by the Bioethics Committee of the Pomeranian Medical University, Szczecin (approval number KB-0012/139/17). All participants gave written informed consent to participate in the study.

Stool sampling

Patients from study and control groups were asked to collect a stool sample into a screw-capped collection container using a plastic holder to use the collection container in the toilet. Study participants were advised not to use laxatives. All study participants were required to collect fecal samples following an overnight fast. Furthermore, hospital patients collected material for testing on the day of admission to the hospital ward or the following day, always before TURP surgery. After the stool was collected, patients stored faeces in refrigerator and delivered the samples within 24 h to our laboratory using ice bags. The samples were then stored at − 80 °C until the analyses.

Microbial genomic DNA extraction from stool sample and quantification of bacterial DNA. DNA extraction from fecal samples was performed using the QIAamp PowerFecal Pro DNA Kit (Cat. No. 51804) according to the instructions of the manufacturer (Qiagen, Germany). Metagenomic libraries encompassing the V3 - V4 hypervariable region of the bacterial 16 S rRNA gene were prepared using the primers 341 F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC)[29]. Next generation sequencing was run using MiSeq platform (paired-end 2 × 250 bp) using V2 chemistry from Illumina (Illumina, San Diego, CA, USA).

16 S sequences processing

Data preprocessing

In this section, we describe the comprehensive methodology employed for processing raw bacterial sequences obtained through 16 S sequencing of the V3-V4 region. The preliminary processing was executed using Qiime2 version 2023.5.

Primer removal

The initial phase of data preprocessing involved the extraction of primer sequences from the raw sequences. This step was carried out using the cutadapt.methods.trim_paired function. The sequences were trimmed in pairs, with the forward primers "CCTACGGGNGGCWGCAG" and "GGATTAGATACCCBDGTAGTC," as well as the reverse primers "GACTACHVGGGTATCTAATCC" and "CTGCWGCCNCCCGTAGG" serving as reference points for trimming. Sequences that did not align with the primers were discarded to ensure the quality and reliability of the subsequent analyses.

Denoising

Subsequent to primer removal, the denoising process was conducted using the dada2.methods.denoise_paired function. This step involved the application of denoising algorithms to the trimmed sequences. Notably, truncation lengths were defined for the forward and reverse reads, specifically 230 bases for the forward reads and 220 bases for the reverse reads. Additionally, a maximum expected error (max_ee) threshold was set at 2 for forward reads and 5 for reverse reads. These parameters were chosen to minimize noise and enhance the accuracy of subsequent analyses.

Feature table and representative sequences filtering

Following denoising, the resulting feature table and representative sequences underwent further refinement. The feature_table.methods.filter_features function was used to filter the feature table, ensuring that only features with a minimum count of 10 across all samples were retained.

Taxonomic classification

The next phase of data processing involved taxonomic classification to assign taxonomic identities to the ASVs within the dataset. To achieve this, the qiime feature-classifier classify-consensus-blast() function was employed. This function utilized the silva 138 SSURef NR99 full-length sequences reference taxonomy database as a point of reference for classification.

The filtered feature table obtained from the previous step was subjected to taxonomic filtering. This involved employing the taxa.methods.filter_table function. The filtering process aimed to exclude any features associated with mitochondria and chloroplasts (exclude="mitochondria, chloroplast"). Furthermore, the taxonomic filtering process was extended to exclude features classified under the the Eukaryota domain (exclude="d__ Eukaryota"). The purpose of these exclusions was to focus the analysis specifically on bacterial taxa and eliminate potential contamination or misclassification from non-bacterial sources. After the exclusion of Eukaryota, a total of 14,560 ASVs remained in the dataset. Following taxonomic filtering, the dataset was collapsed at the genus level. If a taxonomic entry was unassigned or labeled as "uncultured", the nearest properly assigned taxonomic level was selected to represent this ASV.

From this point onward, these taxonomic groups will be referred to as features. As a result, the final dataset contained 317 unique features.

Statistical analysis

All of the statistical analysis was performed in the R environment (version 4.3.1), aimed at elucidating intrinsic patterns within the microbiome data.

Alpha diversity assessment

The evaluation of alpha diversity commenced with ASV abundance table transformation into relative abundances. The diversity function (vegan package) was employed on the raw data to obtain mean values of diversity metrics, including richness, evenness, Simpson, and Shannon indices.

In order to enhance the interpretability of diversity indices, the metrics were further transformed into effective numbers according to the methodology outlined in Lefcheck (2012)[30]. This transformation allows for a more intuitive comparison of diversity by expressing it in terms of the number of equally abundant species that would produce the observed diversity value. Specifically, the Simpson index was transformed using the formula: Effective Simpson = 1/(1-D),

where D represents the Simpson index. The Shannon index was exponentiated exp(H'), providing diversity estimates in effective species numbers.

The Wilcoxon Rank Sums test was used to measure differences in alpha diversity metrics across study and control groups.

Beta diversity analysis

The assessment of beta diversity disparities between control and BPH patients was conducted using the PERMANOVA technique on Bray-Curtis distances, implemented via the *vegan* package, with 9999 permutations. Prior to the analysis, features were filtered based on a 20% prevalence threshold.

	Control group, $n=81$ (%MetS = 44,4)					Study group, $n=76$ (%MetS = 40,8)					
	Mean	Median	Min	Max	SD	Mean	Median	Min	Max	SD	*p*
Age	54,66	54	45	72	6,52	65,79	67	49	79	6,64	< 0,001
Prostate volume (PV) [ml]	22,91	22,1	11,60	39,00	6,19	69,72	60	35,00	240,00	28,22	< 0,001
Qmax [ml/s]	20,08	19,2	6,30	42,10	9,01	10,03	9,2	2,00	26,5	5,33	< 0,001
IPSS	5,14	4,00	0,00	20,00	4,43	18,62	19,00	3,00	35,00	7,80	< 0,001
IPSS (symptoms - I, II, III)	1,20	1,00	1,00	3,00	0,43	2,34	2,00	1,00	3,00	0,72	< 0,001
IPSS-S (storing)	2,70	2,00	0,00	8,00	1,96	8,48	9,00	1,00	15,00	3,55	< 0,001
IPSS-V (voiding)	2,40	1,00	0,00	13,00	2,85	10,14	11,00	0,00	20,00	5,00	< 0,001
QLS	1,43	1,00	0,00	4,00	1,07	3,25	3,00	0,00	5,00	1,23	< 0,001
BMI	28,48	27,6	21,63	38,71	3,79	28,07	28,00	20,06	42,61	3,77	0,616

Table 1. Characteristics of the study groups based on age, BMI, prostate volume, IPSS and QoL questionnaire results.

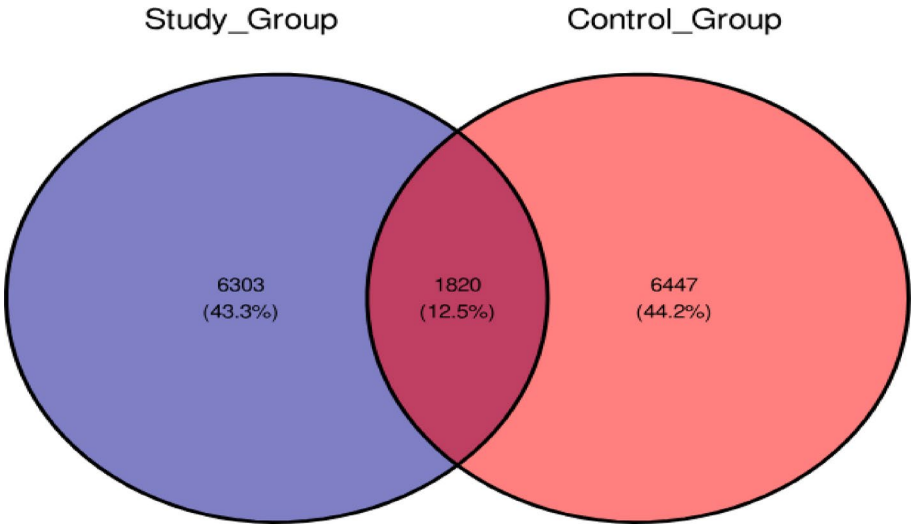

Fig. 1. Number of common ASV between control and study groups. Venn diagram illustrates the quantities of shared and unique ASVs within both groups.

Enterotype determination

The exploration of enterotypes involved the application of the Dirichlet-Multinomial model, executed for selecting community types. This selection process was guided by the Laplace criterion, facilitating the identification of pertinent community configurations within the dataset.

In pursuit of understanding potential distinctions in the prevalence BPH among the distinct enterotypes identified through the earlier employed Dirichlet-Multinomial model, we executed the Fisher Exact Test. This statistical test was employed to determine whether significant differences in the proportions of BPH patients existed across the identified enterotypic clusters.

Results

Group characteristics

The study included 157 men. The control group consisted of 81 healthy men without BPH (mean PV = 22.91 ml ± 6.19, mean Qmax = 20.08 ml/s ± 9.01). The study group consisted of 76 patients diagnosed with BPH who were eligible for transurethral resection of the prostate (TURP) (mean PV = 69.72 ml ± 28.22, mean Qmax = 10.03 ml/s ± 5.33). There were statistically significant differences between the groups (Table 1) for prostate volume ($p < 0.001$), Qmax ($p < 0.001$), IPSS questionnaire scores ($p < 0.001$), QoL ($p < 0.001$). There was also a statistically significant difference in patients' age. However, as previously stated BPH occurs in ageing men predominantly. We consider this as major limitation of our study.

Differences of the fecal microbiota in healthy and BPH patients

Stool samples from 157 men ($n = 81$ control group, $n = 76$ study group) were used to characterise the composition of gut bacteria in terms of similarity. A Venn diagram was used to show the similarities and differences in ASVs between the control and study groups (Fig. 1). As shown in Fig. 1 comparable number of ASVs were exclusive for both groups.

The results indicate that there are no significant differences in the overall alpha diversity between the two groups, as shown by the richness ($p = 0.42$), Shannon ($p = 0.33$), and Simpson ($p = 0.69$) indices. This suggests that both groups have a similar number of taxa and overall microbial diversity when considering both richness and relative abundance. However, a significant difference was observed in evenness ($p = 0.01$), indicating that while the number of taxa may be comparable, their distribution within the community differs between the groups. This suggests that one group may have a more balanced microbial composition, while the other is more dominated by specific taxa (Fig. 2).

Analysis of the beta diversity metrics provided insights into the microbial community dynamics within the context of the control and study groups. Statistical examination using the Permanova test revealed a significant difference between the two groups (F-value = 5.56, p-value < 0.001) (Fig. 3). The analysis was performed on a dataset comprising 157 samples and including 176 different taxa at the genus level. The analysis suggests that the diagnosis of BPH has a significant impact on the diversity of the gut microbiota in ageing men.

Characteristics of four enterotypes of gut microbiota in healthy and BPH patients

In the last part of our analyses, we inspected 4 Enterotypes (Fig. 4). Enterotype 1 is characterised by its predominant association with the ASV identified as g__Blautia, which has a robust assignment strength of 6.10.

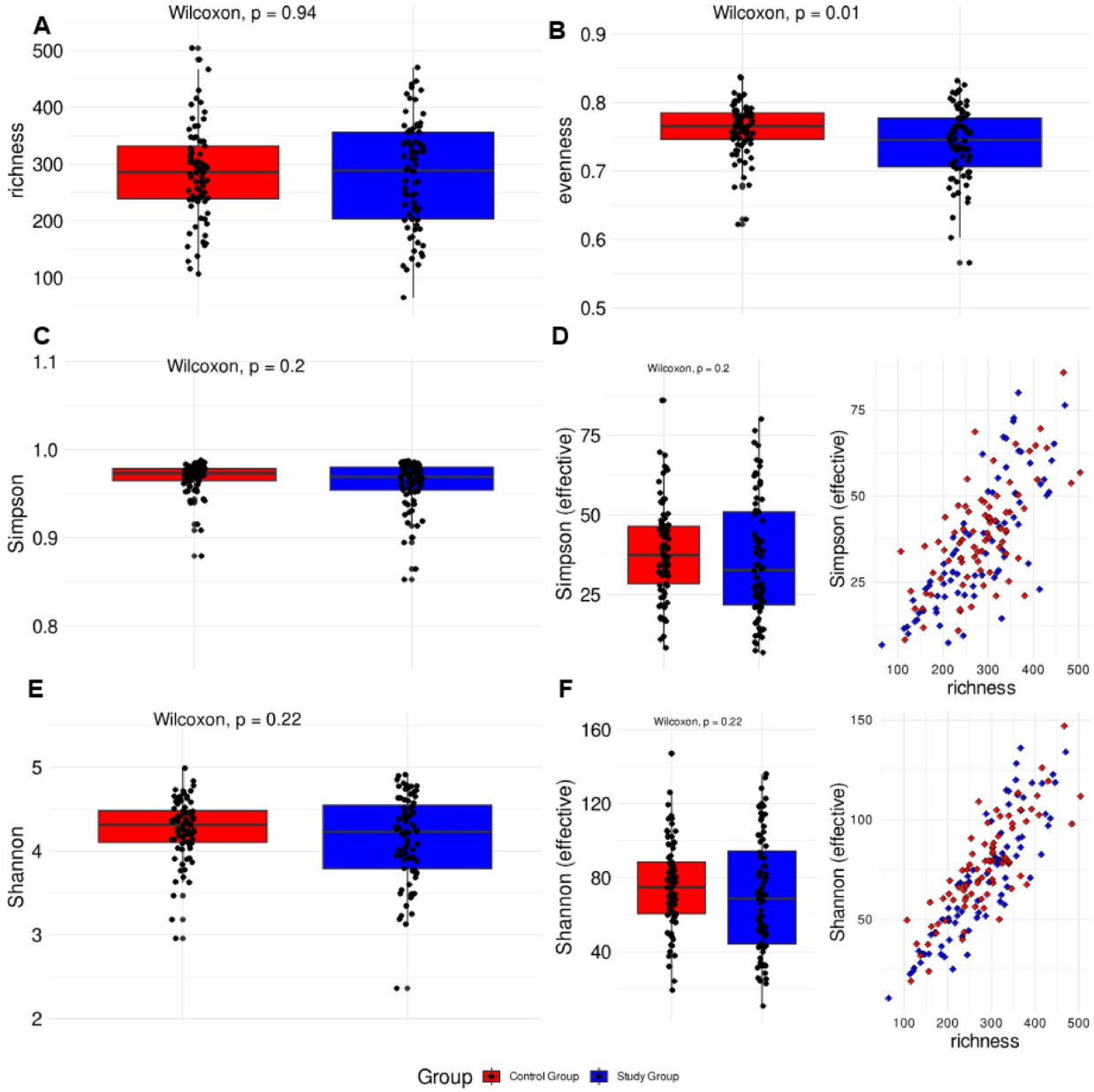

Fig. 2. Alpha diversity comparison of gut microbiota between Control and Study groups, including richness (**A**), evenness (**B**), Simpson (**C**), and Shannon (**E**) indices. Effective values of Simpson (**D**) and Shannon (**F**) were also plotted against richness.

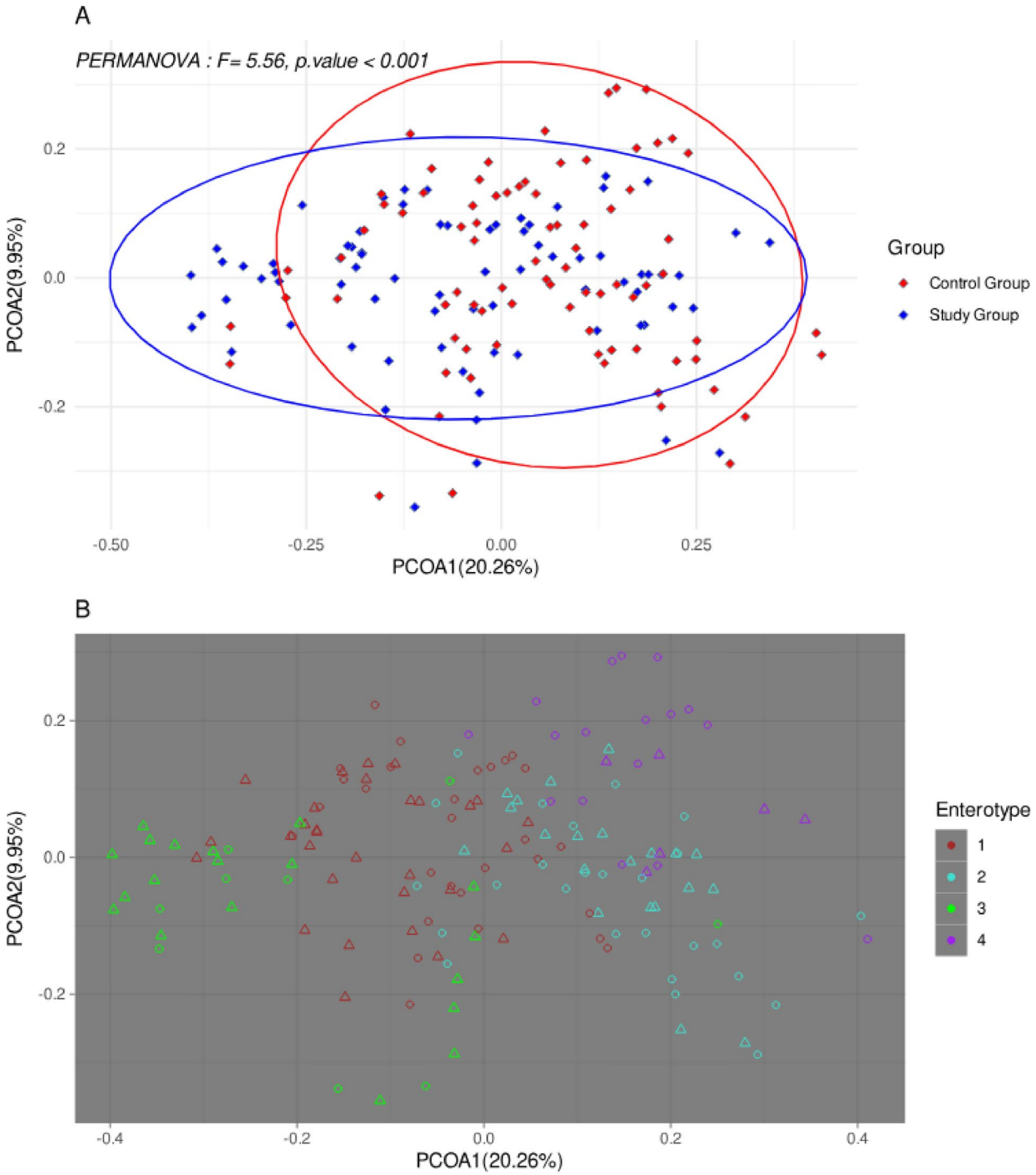

Fig. 3. PCoA plot of Bray-Curtis distance metrics colored by (**A**) group and (**B**) enterotype division. (**B**) - The circles are for control group, and the triangles are the study group.

The substantial presence of this ASV highlights its importance in shaping the microbial composition of this enterotype. In addition, the enterotype contains other notable ASVs, including g__Bacteroides (assignment strength: 3.38) and g__Faecalibacterium (assignment strength: 2.54), which contribute to the microbial configuration of this enterotype.

Enterotype 2 is clearly characterised by the dominance of the g__Bacteroides ASV with a assignment strength of 10.04. In addition, g__Faecalibacterium (assignment strength: 5.54) and g__Blautia (assignment strength: 4.42) are other prominent constituents that together define the distinctive microbial fingerprint of enterotype 2.

Enterotype 3 is characterised by the prevalence of the g__Blautia ASV, which has an assignment strength of 3.51, reflecting its strong influence on the microbial composition of this enterotype. Also noteworthy are g__

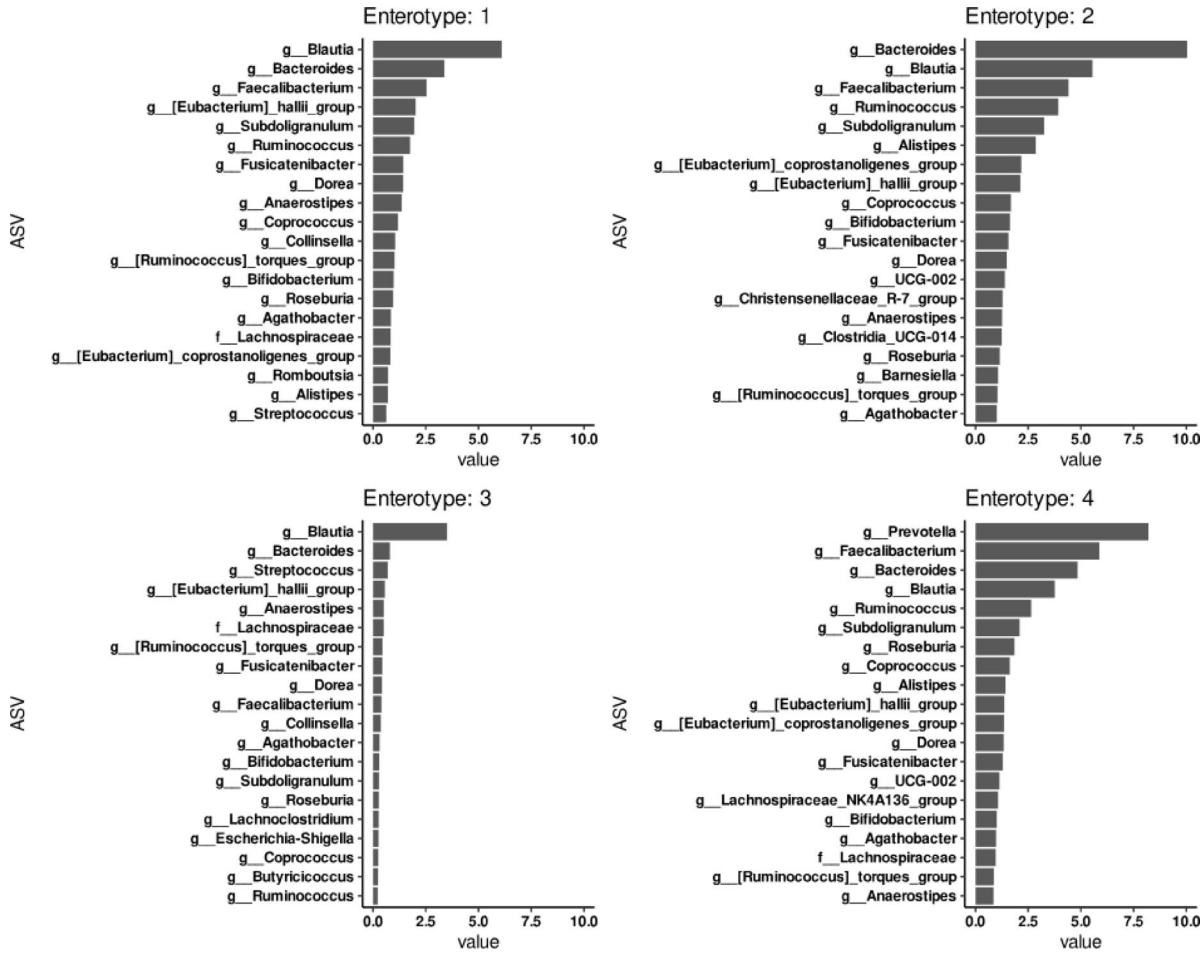

Fig. 4. Microbial composition across distinct enterotypes. The composition was determined by the assignment strength of the strongest ASV within each enterotype. Notably, the delineation of these enterotypes, denoted as Enterotype 1, Enterotype 2, Enterotype 3, and Enterotype 4, emanates from the application of the Dirichlet-Multinomial model.

Bacteroides (assignment strength: 0.79) and f__Lachnospiraceae (assignment strength: 0.7), which contribute to the complex microbial consortium that defines enterotype 3.

Enterotype 4 is emblematic of the g__Prevotella ASV, with an assignment strength of 8.21, which serves as a keystone species shaping the microbial assemblage within this enterotype. In addition, g__Faecalibacterium (assignment strength: 5.87) and g__Bacteroides (assignment strength: 4.84) further complement the microbial landscape and substantiate the distinctive microbial signature of enterotype 4.

Fisher Exact tests were used to measure association between enterotypes and their respective group assignments. The results of these tests were corrected for multiple comparisons using the False Discovery Rate (PFDR).

Aggregation of all enterotypes yielded a PFDR-corrected p-value of 0.035. The other enterotype comparisons are presented in Table 2 and depicted in Fig. 5. The Enterotype 1–3 comparison showed a PFDR corrected p-value of 0.035 and the Enterotype 3–4 analysis yielded a PFDR-corrected p-value of 0.021, with an OR of 0.17 and a 95% CI of (0.04, 0.62).

Discussion

In our study, we analysed differences in the composition of the gut microbiota in men diagnosed with BPH compared to controls (healthy, ageing men). Our study is one of the very few that has analysed the composition of the gut microbiota in patients with BPH. Previous research has mainly focused on the relationship between the gut microbiota and the development of prostate cancer[15,31–33]. These studies have confirmed that inflammatory bowel disease (IBD), which is associated with changes and dysregulation in the composition of the gut microbiota (a state of dysbiosis), increases the risk of prostate cancer[34–38].

In present study, first, we evaluated the similarities of microbial features and diversity of the faecal microbiota of healthy (control group) and BPH patients (study group). We found no significant differences in the alpha diversity. This was not the case for the beta diversity analysis, which confirmed that the difference between the groups was significant (Permanova test: F-value = 5.56, p value < 0.001). A similar observation

	Enterotype			
	1	2	3	4
Control Group (n, %)	35 (57.4%)	20 (48.7%)	9 (29.0%)	17 (70.8%)
Study Group (n, %)	26 (42.6%)	21 (51.3%)	22 (71.0%)	7 (29.2%)
All enterotypes	Fisher test PFDR = 0.035			
Enterotype 1–2	Fisher test P_{FDR} = 0.423, OR = 1.41, CI_{95}(0.59, 2.34)			
Enterotype 1–3	Fisher test P_{FDR} = 0.035, OR = 3.24, CI_{95} (1.19, 9.42)			
Enterotype 1–4	Fisher test P_{FDR} = 0.379, OR = 0.55, CI_{95} (0.17, 1.67)			
Enterotype 2–3	Fisher test P_{FDR} = 0.203, OR = 2.31, CI_{95} (0.785, 7.14)			
Enterotype 2–4	Fisher test P_{FDR} = 0.203, OR = 0.39, CI_{95} (0.11, 1.28)			
Enterotype 3–4	Fisher test P_{FDR} = 0.021, OR = 0.17, CI_{95} (0.04, 0.62)			

Table 2. Comparisons of the prevalence of enterotypes.

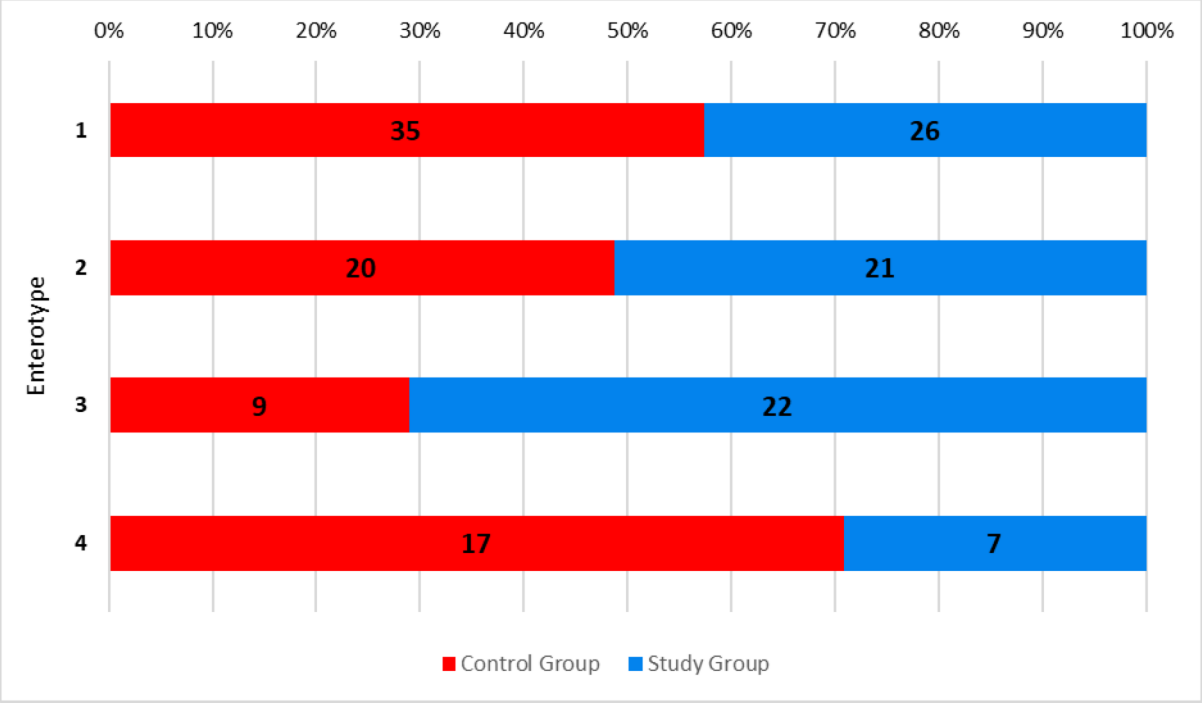

Fig. 5. Percentage distribution of identified enterotypes in the studied population.

was made in a study conducted in a rat model[9]. Authors demonstrated differences in the beta diversity of the gut microbiota were demonstrated between the control group of animals and animals with BPH. It was also found that in the group of animals with BPH there was a significant decrease in Muribaculaceae ($P<0.01$), Turicibacteraceae ($P<0.05$), Turicibacter ($P<0.01$) and Coprococcus ($P<0.01$), while in healthy animals the bacteria Mollicutes ($P<0.05$) and Prevotella ($P<0.05$) were significantly predominant. Another study, also conducted in a rat model with BPH[10] confirmed that the gut microbiota changed with the development of BPH. The study observed that with the induction of BPH, the abundance of bacteria from *Flavonifractor*, *Acetatifactor*, *Oscillibacter*, *Pseudoflavonifractor*, *Butyricimonas* and *Anaerotruncus* genera increased, while the abundance of *Lactobacillus* decreased. There was also an increase in the abundance of genera: *Lactobacillus*, *Anaerobacterium* and *Desulfovibrio* after finasteride therapy, which has a positive effect on preventing the development of BPH, because the presence of these bacteria is associated with the progression of cell apoptosis in the prostate gland[10].

In the study by Takezawa et al.[12], 128 patients were divided into two groups: those with an enlarged prostate (PE, PV ≥ 30 ml) and those without an enlarged prostate (non-PE, PV ≤ 30 ml). Men in the PE group had a higher proportion of Firmicutes and Actinobacteria, while men in the non-PE group had a higher proportion of Bacteroidetes. The Firmicutes/Bacteroidetes (F/B) ratio was significantly higher in the PE group[12]. In a study

by Tsai et al.[39] analysing urine and gut microbiota, no significant differences in gut microbiota composition were observed between patients with BPH and healthy men. The authors of the study indicated that the lack of differences between the intestinal microbiota analyzed using the 16 S rRNA sequencing method may be due to the susceptibility of the intestinal microbiota to change, including the variantions due to antibiotic therapy. However, the discrepancy in results between the study by Tsai et al. and our study is due to the differing exclusion periods for patients who had undergone antibiotic therapy prior to stool sample collection (2 months vs. 6 months). Additionally, in our study we did not analyze the differences between bacterial genera, but focused on the classification and analysis of enterotypes, which are considered more stable and invariable to differentiating factors such as gender, age, and long-term dietary habits. However, in the mentioned study, significant differences in microbiota composition were found between the patients with BPH and control group in urine samples. Differences were mainly noticed in five bacterial genera, namely *Alcaligenes*, *Pseudomonas*, *Lactobacillus*, *Akkermansia*, and *Cetobacterium*[39].

The relationship between gut microbiota and BPH was also investigated by Xia et al.[11], who performed a two-sample Mendelian randomisation (MR) analysis using data from 154.768 individuals (MiBioGen Consortium, FinnGen Consortium). The results suggest that *Eisenbergiella* and Ruminococcaceae (UCG009) have a protective effect against BPH, while *Escherichia shigella* may increase the risk of BPH[11]. Research to date does not provide a clear answer about the composition of the gut microbiota in patients with BPH, but suggests that changes in the composition and function of the microbiota occur. The results so far indicate that further detailed research is needed in this direction and to answer the question of whether the dysfunction of the intestinal microbiota contributes to the development of prostate hyperplasia or, conversely, whether prostate hyperplasia and the associated hormonal changes affect the intestinal microbiota.

In the next step of our study, we defined four groups of different bacterial enterotypes among men from the control and study groups. The overall enterotype distribution between the control and study groups is statistically different ($p = 0.035$), mainly driven by the prevalence of Enterotype 3 in the study group. The comparison between these revealed that the occurrence of enterotype 3 increased the odds for BPH more than three times (OR = 3.24) in comparison to enterotype 1. Also Enterotype 3 was significantly more likely to occur in the study group compared to Enterotype 4, which was more frequent in the control group ($p = 0.021$, OR = 0.17).

Our study is the first one which have attempted to assess the enterotypes in men with BPH and unfortunately, due to the lack of other literature, we cannot compare our results with those of other authors. Overall, our results indicate that the presence of Enterotype 3 might be microbiological pattern of BPH.

Enterotype 3 in present study is dominated by *Blautia*, *Bacteroides* and *Streptococcus*. In patients with ulcerative colitis, Blautia may be involved in the formation of secondary bile acids, which may be involved in the process of carcinogenesis[40]. It is also a species with the ability to produce bacteriocins, which prevent the development of pathogenic bacteria and their colonisation of the intestine[41].

On the other hand, in relation to prostate disease, Holland et al.[13], based on faecal microbiota studies of patients with LUTS, found a significant negative correlation with Lachnospiraceae (Blautia), which increases the bioavailability of SCFAs in the gut. Furthermore, L. Blautia had a protective effect against the symptoms of LUTS and may also influence the severity of the condition[13].

Also, in a study conducted by *Holmberg et al. (2024)*[42], which used human-to-mouse microbiota transplantation and ex vivo analysis of colonic mucus function, the mucus-stimulating capacity of *B. coccoides* strain was confirmed. With dietary fiber supply and *B. coccoides* supplementation, the production of short-chain fatty acids (SCFAs), mainly propionate and acetate, increases. These acids, acting through the specific Ffar2 receptor, increase mucus secretion, which affects the maintenance of mucosal barrier function and provides protection of the intestinal epithelium against infections, inflammation and the effects of toxic factors. In a study conducted by Niu et al.[43] on a murine model, *B. coccoides* was found to have properties regulating metabolic disorders. In another study[44], also conducted on a murine experimental model of lipopolysaccharide (LPS)-induced acute liver injury (ALI), the hepatoprotective effect of *B. producta D4* and *B. producta DSM2950* was confirmed.

Taking all of these into account, we propose that the increase in this genus may be associated with the healing process or a rebound effect in patients.

Streptococus (phylum Firmicutes) was another genus found to be predominant in Enetrotype 3. In the context of prostate disease, it has been confirmed that *Streptococcus* strains are more abundant in rectal swabs from patients with PCa compared to patients without PCa, but no significant differences in the diversity of the gut microbiota have been observed[33]. Other studies confirm that in hCRPC patients with poor clinical outcome, regardless of disease stage, the gut microbiota includes, among others *Streptococcus vestibularis*, *Ruminococcus* sp. DSM_100440, *Ruminococcus* sp. OM05_10BH and *Clostridiales bacterium* VE202_14 [45]. On the other hand, in a study conducted by Sfanos et al. (2018)[32] on 21 men diagnosed with prostate cancer, it was found that *Streptococcaceae* bacteria were less common in men with PCa receiving androgen deprivation therapy than in men with PCa receiving androgen axis targeting therapy. Studies have also demonstrated the presence *of Streptococcus mitis*, but also *Staphylococcus haemolyticus*, *Chlamydia trachomatis*, *Cutibacterium acnes* and Acidovorax sp. in the prostate tissue of a man without obvious LUTS. It was found that the presence of bacteria may contribute to pathological changes in glandular tissue in the early stages of development of benign hyperplasia[46]. In other studies, the bacterial genus Streptococcus (together with the fungal genus Candida) was observed in urine samples from men diagnosed with BPH and severe LUTS[47].

Conclusions

Our study provides new insights into the composition of gut microbiota in men diagnosed with benign prostatic hyperplasia (BPH), with particular focus on enterotype classification. The key findings are as follows:

1. **Microbiota Diversity**: While no significant differences (except evenness) were observed in alpha diversity between the BPH and control groups, beta diversity analysis revealed a significant distinction in gut microbiota composition, aligning with previous animal model studies.
2. **Enterotype Distribution**: The overall distribution of enterotypes between BPH patients and healthy controls was significantly different, driven mainly by the prevalence of Enterotype 3 in the study group. This enterotype, dominated by Blautia, Bacteroides, and Streptococcus, was associated with an increased likelihood of BPH (more than threefold compared to Enterotype 1).
3. **Microbiota-Health Associations**: The increase in Blautia may be linked to healing processes or a rebound effect in patients, given its known anti-inflammatory and probiotic properties. Similarly, the presence of Prevotella, particularly in Enterotype 3, suggests a potential role in metabolic and inflammatory responses, including prostate health. The presence of Streptococcus, though less well understood in BPH, may also be indicative of its involvement in prostate disease.
4. **Gut Microbiota as a Potential Marker**: The findings suggest that changes in the gut microbiota, specifically the presence of Enterotype 3, might serve as a microbiological pattern associated with BPH. This raises the potential for the gut microbiota to be considered as a non-invasive biomarker for BPH risk or progression.
5. **Need for Further Research**: While this study is among the first to assess enterotypes in men with BPH, the results underscore the need for further investigation into the bidirectional relationship between gut microbiota and prostate health. Additional research is required to clarify whether dysbiosis contributes to BPH development or if BPH-related changes in the body affect gut microbiota composition.

Limitations of the study

One of the limitations of the study is that there is an age difference between the study group and the control group. However, BPH prevalence increases with age thus younger population serves as control group. Importantly, we excluded patients who had used antibiotic therapy, antidepressants or glucocorticosteroids within six months before the start of the study. These are factors that disrupt the gut microbiota to a large extent.

Data availability

The sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1221641. The dataset includes raw sequencing reads and associated metadata.

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Author contributions

W.R-Z., M.L. conceived and designed the research W.R-Z., K. S-K. analyzed the data; W.R-Z., M.L., O.S. supervised the recruitment of patients and data collection; W.R., K. S-K., A.L. wrote the paper. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The study was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and approved by the Bioethics Committee of the Pomeranian Medical University, Szczecin (approval number KB-0012/139/17). All participants gave written informed consent to participate in the study.

Additional information

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