ORIGINAL ARTICLE

The gut microbiota associated with high-Gleason prostate cancer

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Abstract

We have found that intestinal bacteria and their metabolites, short-chain fatty acids (SCFAs), promote cancer growth in prostate cancer (PCa) mouse models. To clarify the association between gut microbiota and PCa in humans, we analyzed the gut microbiota profiles of men with suspected PCa. One hundred and fifty-two Japanese men undergoing prostate biopsies (96 with cancer and 56 without cancer) were included in the study and randomly divided into two cohorts: a discovery cohort (114 samples) and a test cohort (38 samples). The gut microbiota was compared between two groups, a high-risk group (men with Grade group 2 or higher PCa) and a negative + low-risk group (men with negative biopsy or Grade group 1 PCa), using 16S rRNA gene sequencing. The relative abundances of *Rikenellaceae*, *Alistipes*, and *Lachnospira*, all SCFA-producing bacteria, were significantly increased in high-risk group. In receiver operating characteristic curve analysis, the index calculated from the abundance of 18 bacterial genera which were selected by least absolute shrinkage and selection operator regression detected high-risk PCa in the discovery cohort with higher accuracy than the prostate specific antigen test (area under the curve [AUC] = 0.85 vs 0.74). Validation of the index in the test cohort showed similar results (AUC = 0.81 vs 0.67). The specific bacterial taxa were associated with high-risk PCa. The gut microbiota profile could be a novel useful marker for the detection of high-risk PCa and could contribute to the carcinogenesis of PCa.

KEYWORDS
bacteria, biomarkers, gastrointestinal microbiome, metagenomics, prostate cancer

1 | INTRODUCTION

Microorganisms are present on every surface of the human body and play a variety of important roles in human health and disease.[1] In particular, the gut microbiota, which is composed of 10^{13} to 10^{14} microorganisms,[2] is the largest and most studied human flora. The gut microbiota is associated not only with local diseases of the intestinal tract, such as inflammatory bowel disease and colorectal cancer,[3,4] but also with systemic diseases such as liver or neurological diseases, suggesting the presence of the so-called gut-liver axis and gut-brain axis.[5,6] Although the bacterial flora present in urine or tissues that are in direct contact with the prostate gland has been suggested to influence local inflammation, hypertrophy, and carcinogenesis, the relationship between the gut microbiota and the prostate is less frequently studied.[7-10]

Prostate cancer is clinically associated with dietary content and nutrients, such as dairy products and fat.[11] The gut microbiota is also strongly influenced by dietary habits and body shape,[12,13] and is involved in host inflammation and immune responses.[14] We have previously shown in animal studies that a high-fat diet and obesity promote local prostate inflammation and PCa proliferation,[15-18] and that SCFAs, major metabolites of intestinal bacteria, promote PCa growth via the IGF-1 signaling pathway.[19] These findings suggest that the gut microbiota, altered by diet and other external factors, may be involved in PCa progression through multiple mechanisms. PCa malignancy is determined by the GG, a specific histopathologic grading system. A higher score, which can range from 1 to 5 points in theory, indicates a higher grade of malignancy. Since the prognosis for patients with GG 1 PCa is usually favorable, overdiagnosis and overtreatment is a problem, and in recent years it has been recommended that most patients with GG 1 PCa should not be treated.[20] On the other hand, some patients with GG ≥2 PCa have a poor prognosis and require prompt and appropriate treatment. Therefore, it

is important to ensure that men with high-grade PCa can be distinguished from men with low-grade cancer. The serum PSA test is the gold standard for PCa screening; however, it cannot distinguish PCa grade. Thus, the development of biomarkers for high-risk PCa is an urgent requirement.

The aim of this study was to examine the association between PCa and the gut microbiota in a Japanese cohort. The gut microbiota profiles of men with or without high-grade PCa were compared to investigate whether the gut microbiota composition could be used as a novel noninvasive marker for high-grade PCa.

2 | MATERIALS AND METHODS

2.1 | Study design

We collected rectal swab samples of 189 Japanese men who were suspected of PCa based on screening methods, such as MRI or PSA tests, and underwent prostate biopsies from December 2018 to March 2020 at the Osaka University Hospital, Osaka Police Hospital, and Osaka General Medical Center. All hospitals are located in Osaka, Japan and patients in the local area were included. The presence or absence of comorbidities was determined by providing medication. Diet, smoking, and alcohol consumption were also assessed with the help of a questionnaire before the prostate biopsy. Men who had used antibiotics within 6 months of sample collection or whose previous antibiotic use was unknown were excluded from the analysis.

Patients who did not have a normal bowel movement, such as diarrhoea, on the day of collection, and patients whose biopsies were discontinued after collection were excluded. In the end, 152 samples were analyzed. These samples were randomly divided into the discovery cohort (114 samples) and the external test cohort (38 samples) for validation (Figure 1). The patients were divided into two
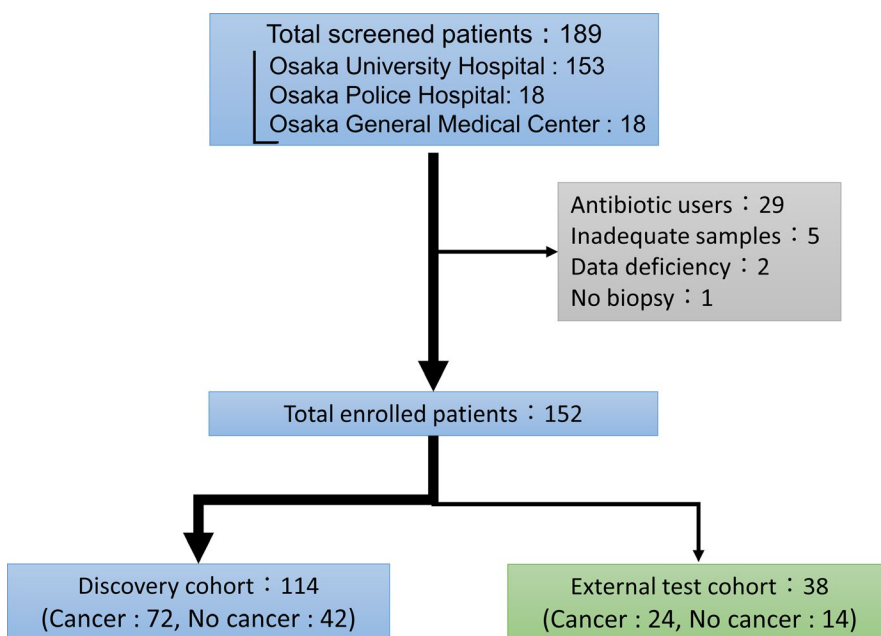

FIGURE 1 Consort flow diagram of cohort composition

groups according to the results of prostate biopsy: a high-risk group (GG 2 or higher PCa) and a negative + low-risk group (negative biopsy or GG 1 PCa). We analyzed all cases using biopsy specimens to evaluate the presence and grade of PCa with uniform criteria.

2.2 | Rectal swab collection

The samples were collected during digital rectal examination before prophylactic antibiotics and prostate biopsy. The rectal examination was performed with sterile gloves, then the finger inserted into the rectum was wiped with a sterile swab, FLOQSwabs (COPAN), taking care not to touch other contaminated areas with the finger or swab. The swab was iced as soon as possible, transported to our laboratory, and stored at −80℃ until bacterial DNA extraction.

2.3 | Bacterial DNA extraction and analysis of the gut microbiota

The samples were suspended in phosphate buffered saline and the bacterial DNA was extracted from the suspension using DNeasy Power Soil Kit (Qiagen). Amplicons targeting the V1-V2 variable regions of the 16S rRNA gene were generated using the primers 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′). Then, a 251-bp paired-end sequencing of the amplicons was performed using a MiSeq (Illumina). The raw sequencing data were processed by the QIIME pipeline version 1.9.1 as the bioinformatics environment. We used PEAR to merge the sequences, and then used UCLUST version 1.2.22q to sort the processed sequences into OTUs with a similarity cut-off of 97%. The annotation of typical sequences of each OTU was performed using RDP Classifier version 2.2 with reference to the Greengenes 13_8 database. Phylogenetic investigation of communities by reconstruction of unobserved states was performed in QIIME to infer the genetic functional profile of the gut microbiota from the OTU composition of each sample. The KEGG pathway abundances were calculated in PICRUSt.[21] The reported nucleotide sequence data are available in the DDBJ Sequenced Read Archive under the accession number DRA011036.

2.4 | Statistical analysis

Comparisons between the two groups were made using the Mann-Whitney U test or chi-square tests. Alpha diversity was assessed by rarefaction analysis. Beta diversity was assessed by PCoA and ANOSIM. Linear discriminant analysis effect size was performed to evaluate significantly different OTUs or functional pathways according to cancer status. During the development of the index for high-risk PCa assessment based on the OTU composition of the samples, variables and formulae were selected and applied, respectively, by the LASSO regression model. The univariate and multivariate analyses were performed by the binomial logistic regression technique. The samples were divided into two groups based on their abundance (higher or lower) with respect to the median abundance of all samples, and the odds ratio for high-risk PCa was calculated for both the groups. Receiver operating characteristic curve analysis was used to calculate the AUC for assessing the discriminatory capacity of PCa. P values less than .05 were considered significant. Rarefaction analysis and PCoA was performed in QIIME, ANOSIM was calculated using R version 4.0.2 package 'Vegan', LEfSe was performed using Galaxy web application (https://huttenhower.sph. harvard.edu/galaxy/), and other statistical tests were performed using JMP Pro 14 (SAS Institute).

2.5 | Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Osaka University (IRB #13397-16) and written informed consent was obtained from all patients.

3 | RESULTS

3.1 | Characteristics of patients from the discovery and external test cohorts

The discovery cohort included 72 men with PCa and 42 men without cancer, and the external test cohort included 24 men with PCa and 14 men without cancer (Figure 1). Radical prostatectomy was performed without preoperative therapy in 23 of 96 PCa patients. There were only three patients whose risk group differed in the surgical pathological data and biopsy specimens. The background characteristics of the discovery cohort are summarized for each GG in Table 1. Age and PSA levels were significantly higher in the high-risk group than in the negative + low-risk group ($P = .0002$ and $P < .0001$). In contrast, there was no difference in BMI, family history, or presence of LUTS among the two groups. The background characteristics of the test cohort are summarized in Table S1. There were no significant differences in age, BMI, PSA, and family history in this cohort. There were no significant differences in lifestyle-related characteristics (smoking, drinking alcohol, diabetes, hypertension, dyslipidemia, and dietary habits) between the two groups in the discovery and test cohort (Tables S2 and S3).

3.2 | The phylogenetic diversity tended to be higher in the gut microbiota of high-risk PCa patients

To assess the alpha diversity of the gut microbiota, the richness and evenness were evaluated based on the PD and Shannon index, respectively (Figure 2A and Figure S1A). There was no significant difference in the Shannon index not only between the negative + low-risk and high-risk groups (Figure 2B, $P = .1667$), but also

TABLE 1 Background characteristics of the discovery cohort

Grade group	Negative + low risk		High risk				P-value[a]
	No cancer	1	2	3	4	5	
Cases	42	14	21	11	18	8	
Age year median (quartile)	67.0(62.0–71.8)	66.5 (62.0–72.8)	73.0 (68.0–76.0)	71.0 (70.0–74.5)	70.5 (65.8–73.0)	73.5 (72.0–76.8)	.0002
BMI kg/m² median (quartile)	24.8 (22.0–25.8)	23.7 (20.2–24.7)	24.0 (22.8–25.0)	22.6 (22.2–23.3)	23.4 (21.0–26.1)	23.4 (21.6–26.4)	.5824
PSA ng/mL median (quartile)	7.0 (4.6–9.9)	6.2 (5.0–8.9)	8.1 (6.0–12.4)	14.4 (8.5–21.5)	11.7 (6.8–68.7)	177.2 (50.7–360.0)	<.0001
<4 (%)	3 (7.1)	1 (7.1)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)	
4-10 (%)	28 (66.7)	10 (71.5)	14 (66.7)	2 (18.2)	7 (38.9)	0 (0.0)	
>10 (%)	11 (26.2)	3 (21.4)	7 (33.3)	8 (72.7)	11 (61.1)	8 (100.0)	
Family history (%)							.7337
+	1 (2.4)	2 (14.3)	1 (4.8)	1 (9.1)	2 (11.1)	0 (0.0)	
−	38 (90.5)	11 (78.8)	19 (90.4)	10 (90.9)	14 (77.8)	7 (87.5)	
Unknown	3 (7.1)	1 (7.1)	1 (4.8)	0 (0.0)	2 (11.1)	1 (12.5)	
LUTS (%)							.8903
+	8 (19.0)	3 (21.4)	2 (9.6)	2 (18.2)	6 (33.3)	2 (25.0)	
−	33 (78.6)	11 (78.6)	19 (90.4)	8 (72.7)	12 (66.7)	6 (75.0)	
Unknown	1 (2.4)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)	
clinical T stage (%)							
1c		4 (28.6)	3 (14.3)	2 (18.2)	1 (5.6)	0 (0.0)	
2		9 (64.3)	16 (76.2)	8 (72.7)	7 (38.8)	2 (25.0)	
≥3		1 (7.1)	2 (9.5)	1 (9.1)	10 (55.6)	6 (75.0)	
Metastasis (%)							
+		0 (0.0)	1 (4.8)	2 (18.2)	5 (27.8)	5 (62.5)	
−		14 (100.0)	20 (95.2)	9 (81.8)	13 (72.2)	3 (37.5)	

Abbreviations: BMI, body mass index; LUTS, lower urinary tract symptoms; PSA, prostate-specific antigen.

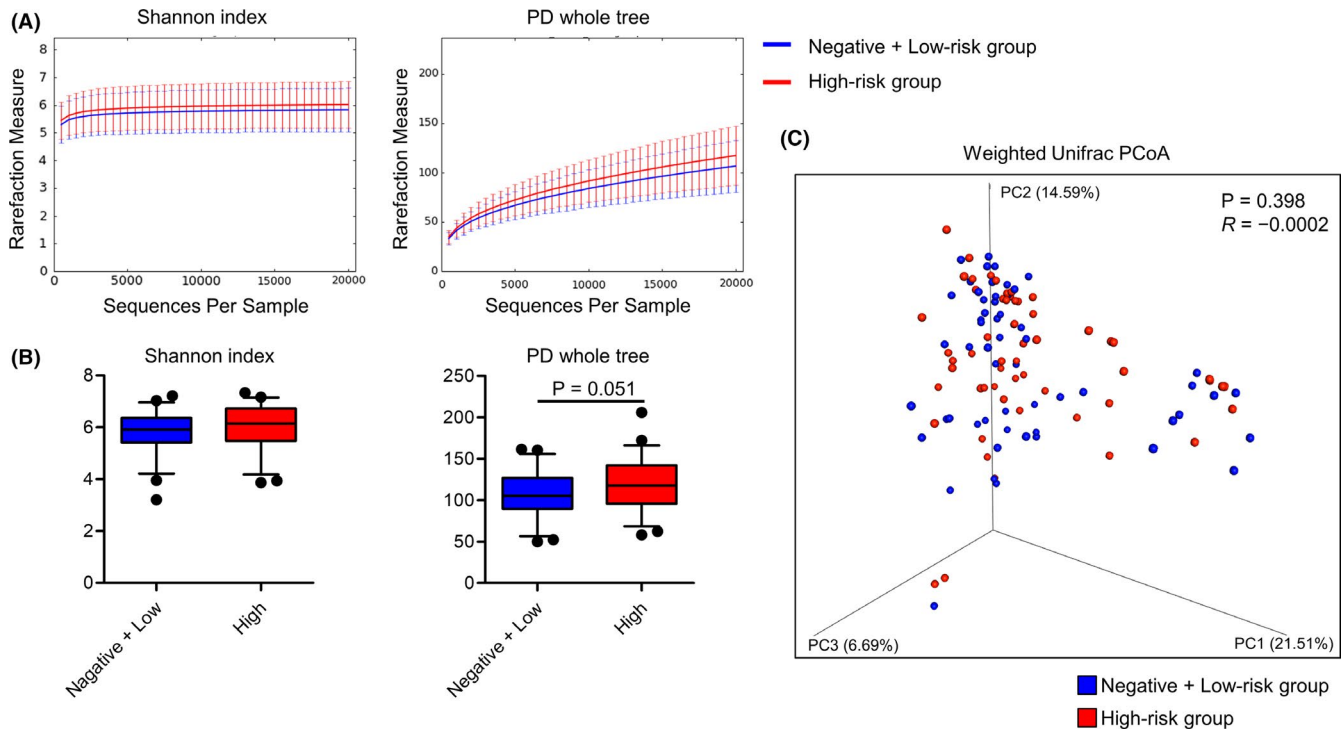[a]Negative + low risk versus high risk.

FIGURE 2 Alpha and beta diversity of the gut microbiota in the discovery cohort. A, Rarefaction analysis of the Shannon index (left) and phylogenetic diversity (PD) whole tree (right). The data are presented as mean ± standard deviation. B, Boxplots depicting the Shannon index (left) and PD whole tree (right) at 20 000 sequences. The data represent median (line in box), interquartile range (box), and 5–95 percentiles (whiskers). C, Principal coordinate analysis plots based on weighted UniFrac distance showing gut microbiota composition in the discovery cohort. The blue line and dots represent the negative + low-risk group, and the red line and dots represent the high-risk group

between men with and without PCa (Figure S1B, $P = .2484$). The PD tend to be higher in the high-risk group than in the negative + low-risk group (Figure 2B, $P = .0515$). This trend was the same when comparing men with and without PCa (Figure S1B, $P = .0673$). No significant differences were identified in the beta diversity between the negative + low-risk and high-risk groups (Figure 2C, $P = .398$, $R = -.0002$). Similarly, there was no significant difference in beta diversity between men with or without PCa (Figure S1C, $P = .115$, $R = .034$). The PCoA showed an obvious overlap of the bacterial component profile between both groups (Figure 2C).

3.3 | Specific bacteria and metabolic pathways were significantly increased in the gut microbiota of men with high-grade PCa

From the phylum to the genus level, nine OTUs, identified in this analysis, had significantly higher relative abundance in the gut microbiota of the high-risk group ($P < .05$, LDA score > |2.0|) (Figure 3A). Two of the nine OTUs could not be annotated. There was a particularly remarkable difference in the abundance of *Rikenellaceae*, *Alistipes* and *Lachnospira* (LDA score > |2.5|). Similarly, the abundance of these three bacteria was significantly higher in patients with PCa (LDA score > |2.5|) than that in men without cancer (Figure S2A). There was no significant difference in the abundance of each of the three bacteria between PCa patients with and without metastases (Figure

S2B). An analysis of the gut microbiota functional profile significantly differed between the negative + low-risk and high-risk PCa groups ($P < .05$, LDA score > |2.0|) (Figure 3B), which contained five metabolic pathways (starch and sucrose metabolism, phenylpropanoid biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, cyanoamino acid metabolism, and histidine metabolism). Similarly, these five metabolic pathways were significantly more common in the patients with PCa than in the men without cancer (Figure S2C).

3.4 | Fecal microbiome prostate index could identify patients with high-grade PCa

We examined whether the gut microbiota profile could identify high-risk PCa in the test cohort. Each of the three highly abundant bacteria in the patients with high-risk PCa (*Rikenellaceae*, *Alistipes*, and *Lachnospira*) showed low accuracy in identifying men with high-risk PCa when validated in the test cohort (AUC = 0.62, 0.59, and 0.54) (Figure 4A). Thus, we used the LASSO regression model to perform a variable selection of 503 OTUs, excluding 221 OTUs that were not annotated and whose bacterial name could not be identified, and developed the index for high-risk PCa assessment based on the OTU composition (Figure 4B). Eighteen OTUs were identified that were particularly associated with the high-risk group (Table 2). A regression equation with the relative abundance of these 18 bacteria was calculated, and the
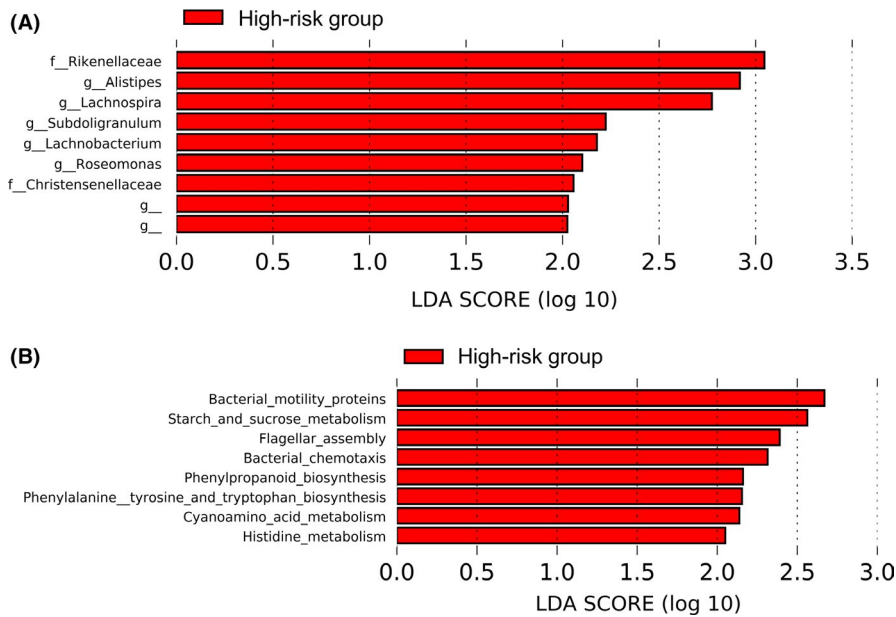
(A)

(B)

FIGURE 3 Comparison of the abundance of operational taxonomic units (OTUs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways based on cancer grade. A and B, Linear discriminant analysis (LDA) effect size analysis including OTUs (A) and KEGG pathways (B) that were significantly different in abundance between the high-risk and negative + low-risk groups (*P* < .05 and LDA score > |2.0|). Red bars represent OTUs and pathways positively associated with the high-risk group. The bars without bacterial names refer to OTUs that could not be identified

value was defined as the FMPI (Appendix S1). The FMPI was significantly higher in the high-risk group in both the discovery and test cohorts (*P* < .001 and *P* = .001) (Figure 4C). When comparing the existing risk factors in the univariate logistic regression analysis, PSA levels, age, and FMPI were shown to be significant factors for high-risk PCa. After adjusting for PSA levels and age, the multivariate analysis showed that FMPI remained as a significant risk factor for high-risk PCa (odds ratio = 7.06, 95% CI =2.83-17.66, *P* < .0001) (Table 3). In the discovery cohort, ROC curve analysis showed the accuracy of FMPI at detecting high-risk PCa in men undergoing a prostate biopsy and that the FMPI had higher AUC than PSA levels (AUC = 0.85 vs 0.74) (Figure 4D). Two cut-off points were set up, one for good sensitivity (≥80%) and another for good specificity (≥80%). A 0.47 cut-off resulted in a sensitivity of 0.81, specificity of 0.66, PPV of 0.71, and NPV of 0.77. Then, a 0.51 cut-off resulted in a sensitivity of 0.71, specificity of 0.80, PPV of 0.79, and NPV of 0.73. Validation in the external test cohort showed that FMPI had higher AUC than PSA (AUC = 0.81 vs 0.67) (Figure 4E). A cut-off of 0.47 (good sensitivity in the discovery cohort) resulted in a sensitivity of 0.79, a specificity of 0.63, PPV of 0.68, and NPV of 0.75. A cut-off of 0.51 (good specificity in the discovery cohort) resulted in a sensitivity of 0.63, a specificity of 0.84, PPV of 0.80, and NPV of 0.70. The accuracy of FMPI at discriminating the PCa presence was demonstrated in the discovery (AUC = 0.78) and test (AUC = 0.70) cohorts (Figure S3).
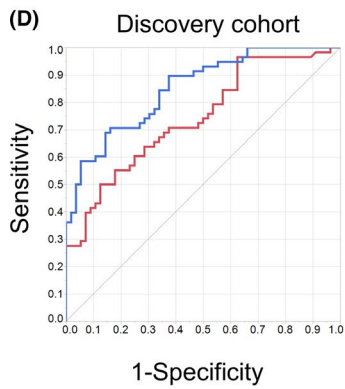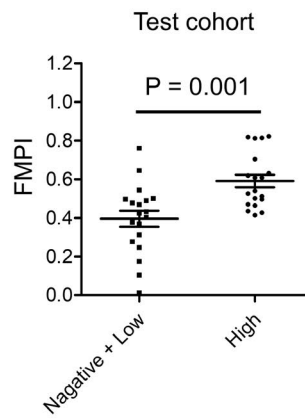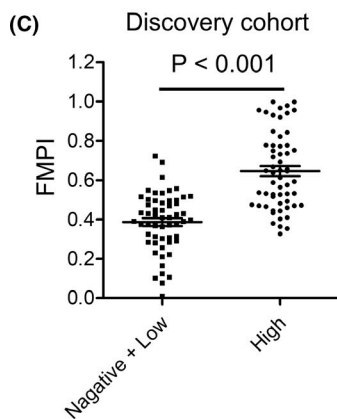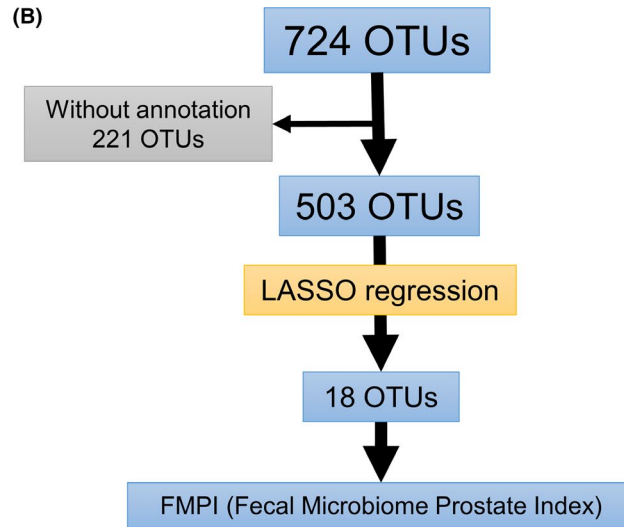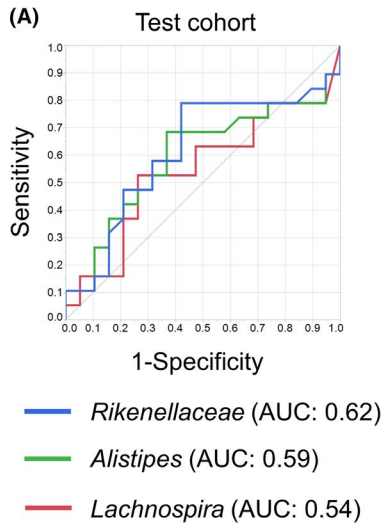
4 | DISCUSSION

In this study, we analyzed the gut microbiota of men suspected of PCa and found that specific bacteria and bacterial metabolic functions were increased in high-risk PCa patients. In addition, we suggested that the gut microbiota composition could be a more accurate predictor of high-risk PCa than the PSA test.

A limited number of studies, mostly on Caucasian cohorts from the USA, have reported the association between PCa and gut microbiota. We hypothesized that the results likely differ between cohorts due to regional and racial differences in the gut microbiota.[22,23] Therefore, we decided to investigate the relationship between gut microbiota and PCa in Japanese men. Additionally, since the gut microbiota is affected by the lifestyle of the host, which includes a variety of factors such as smoking, diet, and medication,[24] this analysis was limited to men living in an urban area to reduce regional differences in lifestyle. Since the Japanese have a unique gut microbiota[22] and no comprehensive gut microbiota analysis of Japanese PCa patients using next-generation sequencers has been reported yet, this study is likely to provide novel insights from these unique cohorts.

This study showed no significant differences in both alpha and beta diversity according to cancer grade, but the PD, which provides an estimate of the richness of the bacterial flora in alpha diversity, tended to be higher in men with PCa than in men without cancer. In contrast, a study in the American population reported that the

FIGURE 4 Validation of the ability of the gut microbiome profile to predict high-risk prostate cancer (PCa). A, Receiver operating characteristic (ROC) curve analysis of *Rikenellaceae* (blue line), *Alistipes* (green line), and *Lachnospira* (red line) for predicting high-risk PCa in the test cohort. B, Consort flow diagram of the fecal microbiome prostate index (FMPI) definition. From the 724 operational taxonomic units (OTUs) in the discovery cohort, least absolute shrinkage and selection operator regression analysis selected 18 OTUs that had a strong correlation with either the presence or absence of high-risk PCa and developed a regression equation for high-risk PCa detection, which was named FMPI. C, Dot plots depicting FMPI values in the negative + low-risk and the high-risk groups in the discovery (left) and test (right) cohorts. D and E, ROC curve analysis and area under the curve of FMPI (blue line) and prostate-specific antigen level (red line), and cut-off points of the FMPI for predicting high-risk PCa in the discovery (D) and test (E) cohorts

(A) Test cohort

Sensitivity vs 1-Specificity

— *Rikenellaceae* (AUC: 0.62)
— *Alistipes* (AUC: 0.59)
— *Lachnospira* (AUC: 0.54)

(B)

724 OTUs

Without annotation 221 OTUs

503 OTUs

LASSO regression

18 OTUs

FMPI (Fecal Microbiome Prostate Index)

(C) Discovery cohort

P < 0.001

FMPI — Nagative + Low / High

Test cohort

P = 0.001

FMPI — Nagative + Low / High

(D) Discovery cohort

Sensitivity vs 1-Specificity

— FMPI (AUC: 0.85)
— PSA (AUC: 0.74)

Cut-off points of FMPI
(sensitivity ≥ 0.8 or specificity ≥ 0.8)

Cut-off	Sensitivity	Specificity	PPV	NPV
0.47	0.81	0.66	0.71	0.77
0.51	0.71	0.80	0.79	0.73

(E) Test cohort

Sensitivity vs 1-Specificity
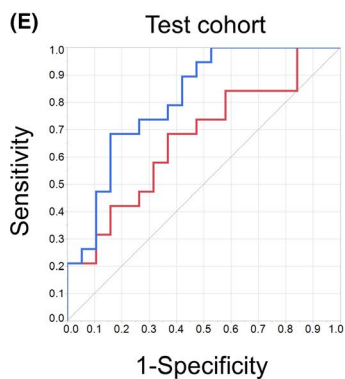
— FMPI (AUC: 0.81)
— PSA (AUC: 0.67)

Cut-off points of FMPI

Cut-off	Sensitivity	Specificity	PPV	NPV
0.47	0.79	0.63	0.68	0.75
0.51	0.63	0.84	0.80	0.70

PD was significantly higher in men without PCa.[25] This difference in alpha diversity trend may reflect regional variations in the gut microbiota profile due to racial and lifestyle diversity. However, multiple other studies in the USA have not found differences in the alpha diversity between men with or without PCa.[26,27] Thus, it should be noted that the association between gut microbiota diversity and PCa is controversial.

In the present study, *Rikenellaceae*, *Alistipes*, and *Lachnospira* were more common in patients with high-risk PCa. These results are quite different from the four PCa-promoting taxa (*Pseudomonas*, *Escherichia*, *Acinetobacter*, and *Propionibacterium*) in prostate tissue flora of the Asian cohort.[8] This suggests that the intestinal bacteria would be different from the prostatic bacteria, and each is involved in PCa growth through different mechanisms. *Alistipes* is a genus belonging to the *Rikenellaceae* family and has been associated with CRC.[28-30] In a multi-cohort analysis, the abundance of *Alistipes* spp. was increased in the gut microbiota of CRC patients in several countries. Evidence shows that in an animal study, the transfer of *Alistipes* induced colitis and promoted colon carcinogenesis via the IL-6 signaling pathway.[28,31] We previously reported that prostate inflammation and activation of IL-6 signaling are involved in the high-fat diet-induced acceleration of PCa growth in an animal model.[15] *Alistipes* may induce inflammation not only in the gastrointestinal tract but also systemically, which may increase the risk of PCa. On the other hand, *Alistipes* is one of the SCFA-producing bacteria, and

previous studies have been focused on its anti-inflammatory function via SCFAs.[32,33] In addition, *Lachnospira*, which is a genus of the family *Lachnospiraceae*, is also an SCFA-producing bacteria.[34] This family is generally considered to be beneficial bacteria for the host because of the anti-inflammatory effects on the intestinal tract and protects us against CRC.[35,36] However, based on the results of this study, these SCFA-producing bacteria, *Alistipes* and *Lachnospira*, could be potential PCa promoters. Sims et al analyzed the gut microbiota of cervical cancer patients and reported that *Alistipes* and *Lachnospira* are less common in the patients.[37] In contrast, we showed that *Alistipes* and *Lachnospira* were markedly increased in the gut microbiota of high-grade PCa patients, suggesting a unique mechanism whereby SCFAs promote PCa. In a PCa mouse model, we have reported that oral administration of antibiotics inhibits cancer growth, and SCFAs from intestinal bacteria play an important role in this PCa progression via the IGF-1 signaling pathway.[19] In addition, *Rikenellaceae* and *Lachnospiraceae* have been identified as potential PCa promoters in mouse gut microbiota, which is similar to the profile in the human microbiota in this study. These results indicate that bacteria-derived SCFAs in the human intestinal tract might also promote PCa progression, as seen in mouse.

Furthermore, the other bacteria that were increased in the patients with high-grade PCa included *Subdoligranulum*, *Lachnobacterium*, and *Christensenellaceae*, which, like *Lachnospira*, belong to the SCFA-producing order *Clostridiales*. These results suggest that SCFAs may play a significant role in PCa progression. *Alistipes* and *Subdoligranulum* are increased in the gut flora of people with a high-quality diet, which includes the intake of more whole fruits and less added sugar, calculated by healthy eating index-2005 in the USA.[38] Therefore, the type of diet and lifestyle that increase these bacteria, which are common in Japanese men with PCa, need to be investigated. Most studies in the USA found an increase in *Bacteroides* spp. in men with PCa.[26,27,39] These results differ from those of our study, suggesting the existence of a unique profile behind PCa development in Japanese men.

Although each bacterial species has a different functional profile, some functions are common among several species. Therefore, it is important to compare not only bacterial compositions, but also bacterial functions. We used PICRUSt to predict a functional profile of the gut microbiota based on the composition of the OTUs identified by 16S rRNA gene sequencing. Starch and sucrose metabolism, the KEGG metabolic function that was most elevated in

TABLE 2 The bacterial taxa selected in LASSO regression analysis

Positive correlation with high-risk PCa	Negative correlation with high-risk PCa
Rosemonas	*Propionspora*
Syntrophococcus	*Sebaldella*
Kyotococcus	*Kocuria*
p-75-a5	*Moryella*
Aeromonas	*Anaerofilum*
Raoultella	*Atopobium*
Eggerthella	*Peptostreptococcus*
Lachnospira	*Blautia*
Phascolarctobacterium	*Acidaminococcus*

Abbreviations: LASSO, least absolute shrinkage and selection operator; PCa, prostate cancer.

TABLE 3 Univariate and multivariate analysis to determine the independent predictor of high-risk PCa

Parameter	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age	4.04	1.85-8.83	.0003	3.89	1.56-9.73	.0036
BMI	0.57	0.27-1.19	.1352	—	—	—
PSA	4.00	1.83-8.71	.0005	3.49	1.41-8.63	.0068
FMPI	7.18	3.22-16.9	<.0001	7.06	2.83-17.66	<.0001

Abbreviations: BMI, body mass index; FMPI, fecal microbiome prostate index; PCa, prostate cancer; PSA, prostate-specific antigen.

the gut microbiota of high-risk PCa patients in this cohort, was also markedly elevated in American PCa patients in a previous study.[26] Although the bacteria increased in the American patients were different from those identified in the current study on Japanese patients, there were common characteristics of metabolic functions in both the Japanese and American cohorts, suggesting that specific bacterial metabolites, rather than specific bacteria, are involved in PCa worldwide. Notably, the pathways involved in multiple types of amino acid metabolism were elevated in the high-risk group. PCa patients have a different profile of amino acids in their blood or urine from that of healthy controls, which may be due to differences in intestinal bacteria.[40-42]

Although we could not accurately detect high-risk PCa based on the relative abundance of a single bacterium, the FMPI, which was calculated from the abundance of the selected bacteria strongly associated (either positively or negatively) with high-risk PCa and could detect high-risk PCa with greater accuracy than the PSA test. These 18 statistically selected bacteria contained nine genera that were increased in high-risk PCa, including not only the above-mentioned *Lachnospira*, but also *Aeromonas* and *Phascolarctobacterium*, which are also increased in CRC patients.[43,44] In addition, the abundances of *Eggerthella*, which was also among the nine genera with higher abundance in the high-grade PCa group, and *Alistipes* in the gut are related to vegetable intake.[45] Therefore, the increased presence of *Eggerthella* likely reflects a scenario similar to that of *Alistipes* in the gut microbiota, indicating that a diet high in fibre, the source of SCFAs, increases the risk of high-grade PCa. On the other hand, *Blautia*, which was among the nine genera with higher abundance in the negative + low-risk group, has been reported in the USA to be inversely associated with the cancer grade in PCa patients.[46] Another genus, *Anaerofilum*, is more common in breast cancer patients with low counts of tumor-infiltrating lymphocytes,[47] suggesting that bacterial modulation of inflammation in distant organs may also influence the progression of PCa. Our findings suggested that PCa is influenced by specific gut bacterial groups through multiple mechanisms, although a single bacterium was not strongly associated with PCa (Table 4). Furthermore, this analysis identified some bacterial genera that have also been shown to be involved in several cancer types in Caucasian cohorts. Since the FMPI could detect high-risk PCa with high accuracy even in the external test cohort, the FMPI could be a very useful marker for high-risk PCa, which needs definitive therapy. Similar results have not been reported in the past, which may be because this study was the first to solely focus on the gut microbiota profile associated with high-risk PCa. Analyzing the gut microbiota and calculating the FMPI using swab samples can be performed as an additional noninvasive test for men suspected of PCa at the time of digital rectal examination, which is an essential test for PCa screening. Therefore, this noninvasive and informative analysis of the gut microbiota might be widely used as an essential test for screening patients with high-risk PCa.

There are several limitations to this study. First, the study cohort was composed only of Japanese men living in an urban area with similar lifestyles. The bacterial composition at risk for PCa differed from the results reported in predominantly Caucasian populations.[26,27,39] Whilst our results have compared the gut microbiota in a cohort with similar lifestyles, our findings may not be translatable to a wider global population. Future validation in a larger cohort with various lifestyles is desirable. Second, we assessed lifestyle based on a brief questionnaire. As such, we were able to confirm that there was no obvious lifestyle bias in our results. However, this study did not allow us to examine the link between PCa and gut microbiota under the influence of lifestyle (eg, dairy intake). A detailed lifestyle assessment should be conducted in future studies. Third, since we used 16S rRNA gene sequencing to analyze the gut microbiota, we could not know the composition at the species level and the actual genetic functions of the microbiota. Shotgun metagenomic sequencing is necessary to elucidate these aspects, but it is costly and requires large amounts of bacterial DNA. In contrast, 16S rRNA gene sequencing was more suitable for analyzing a large number of swab samples with small amounts of DNA. Fourth, because this study was conducted in all men who had been suspected of PCa by PSA test, it is possible that the cohorts without PCa may have a different microbiota than healthy individuals with normal PSA levels. Further prospective studies should be carried out to examine whether the FMPI can be used to assess PCa risk in healthy men. Finally, we could not conclude whether alterations in the gut microbiota of men with PCa were the cause or the consequence of their PCa. Therefore, it is not possible to determine from these results whether intervention of the gut microbiota using probiotics or other agents can reduce PCa risk and development. However, we found that PCa does not alter the gut microbiota in a PCa mice model, and therefore gut bacteria are likely involved in PCa development.[19]

In this study, *Rikenellaceae*, *Alistipes*, and *Lachnospira* were more abundant in the gut microbiota of patients with high-risk PCa. We found that the FMPI estimated from the abundance of 18 specific gut bacterial genera could detect high-risk PCa in Japanese men undergoing prostate biopsy with greater accuracy than the PSA test. These findings suggest the existence of "gut-prostate axis" mediated by specific bacteria and shed lights on the new mechanisms of PCa growth. In recent years, efforts have been made to treat or prevent

TABLE 4 The possible roles of intestinal bacteria associated with the risk of high-grade PCa

SCFAs production	Regulation of inflammation
Alistipes	*Alistipes* (pro-inflammation)
Rikenellaceae	*Anaerofilum* (anti-inflammation)
Lachnospira	
Subdoligranulum	
Lachnobacterium	
Christensenellaceae	
Eggerthella	

Abbreviations: PCa, prostate cancer; SCFAs, short-chain fatty acids.

various diseases by probiotics or other agents; however, in PCa, the influence of gut microbiota has not been well understood until now.[14] Further studies might allow us to use the abundance of the three PCa-associated taxa and the FMPI as a predictive marker for the treatment and prevention of high-risk PCa using bacterial agents.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.