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ORIGINAL ARTICLE

Comparative analysis of gut microbiota in hormone-sensitive and castration-resistant prostate cancer in Japanese men

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Abstract

Gut microbiota plays a crucial role in the development and progression of prostate cancer, with previous studies indicating that certain bacterial taxa are more abundant in castration-resistant prostate cancer (CRPC) compared to hormone-sensitive prostate cancer (HSPC). Notably, the composition of gut microbiota can vary significantly by geographic region, and Japanese individuals have a distinct microbial profile. However, research exploring these differences within Japanese populations remains limited. This study investigated the gut microbiota differences between Japanese men with HSPC and CRPC and further validated these findings using a transgenic mouse model. Rectal swab samples were collected from 140 Japanese men diagnosed with HSPC ($n = 84$) or CRPC ($n = 56$) between September 2020 and July 2022. Gut microbiota composition was analyzed using 16S rRNA gene sequencing. Additionally, *Pten*-KO mice, which model the progression from HSPC to CRPC, underwent similar microbiota analysis. Results revealed significant differences in gut microbiota composition between HSPC and CRPC patients. Specifically, the CRPC group showed a higher abundance of Firmicutes, including *Gemella* and *Lactobacillus*, compared to the HSPC group. These differences were mirrored in the mouse model, where CRPC mice also showed an increase in these bacteria. This study identifies distinct microbial differences between HSPC and CRPC in Japanese men, suggesting that *Gemella* and *Lactobacillus* may be associated with the progression to castration resistance in prostate cancer. These findings suggest that gut microbiota differences may be associated with prostate cancer

Abbreviations: ADT, androgen deprivation therapy; ANOSIM, analysis of similarities; ATT, androgen axis targeted therapy; ARAT, androgen receptor axis-targeted therapy; CAB, combined androgen blockade; CNPC, noncastrated prostate cancer; CRPC, castration-resistant prostate cancer; HFD, high-fat diet; HSPC, hormone-sensitive prostate cancer; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; mCRPC, metastatic CRPC; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; PSA, prostate-specific antigen; SCFA, short-chain fatty acid.

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progression. Further research is needed to explore the potential of targeting the microbiota as a therapeutic strategy.

KEYWORDS
CRPC, *Gemella*, gut microbiota, HSPC, Japanese, *Lactobacillus*, prostate cancer, *Ruminococcus*

1 | INTRODUCTION

In recent years, the gut microbiota has been reported to affect various types of cancers.[1] The gut microbiota varies according to race, region, genetic background, lifestyle, and diet.[2] An increasing body of evidence revealed a bidirectional interaction between the gut microbiota and prostate cancer. An HFD induces gut dysbiosis, and gut microbial metabolites, including SCFAs, promote prostate cancer growth in mice model.[3] Lipopolysaccharide derived from dysbiotic gut microbes stimulates mast cells, resulting in systemic and local inflammation, which in turn promotes prostate cancer growth in HFD-fed mice.[4] Certain abundant gut microbes have been identified in patients with high-risk prostate cancer compared with controls, including healthy volunteers and patients with benign prostatic disease or negative prostatic biopsy results.[5–7] The gut microbiota plays an important role in the progression of CRPC, and tumor progression. Significant compositional differences and greater abundance of specific microbes such as *Akkermansia muciniphila* and Ruminococcaceae spp. were found in the gut microbiota of men taking oral ATT such as bicalutamide, enzalutamide, and abiraterone acetate, and in functional analyses, the pathways involved in steroid biosynthesis and steroid hormone biosynthesis were enriched in the oral ATT group.[8] Gut microbes, affecting androgen biosynthesis, were enriched in the gut microbiota of patients with CRPC taking ARAT.[9–11] In the CRPC mouse model, commensal gut microbes capable of converting dehydroepiandrosterone (DHEA) to testosterone were enriched. The ablation of these microbes by antibiotics delayed CRPC progression in mice.[12] Targeting the gut microbiota and metabolic pathways involved in microbial biosynthesis could be a new therapeutic option for advanced prostate cancer. Considering the aforementioned findings, androgenesis by gut microbiota should be considered in patients undergoing ADT with/without ARAT. It is a well-established fact that the prevalence of prostate cancer differs based on race. Additionally, the gut microbiota is significantly influenced by race and lifestyle, suggesting that comparisons of the gut microbiota between HSPC and CRPC in Asian cohorts differ from existing data. However, very few studies have used Asian cohorts. In this study, we aimed to elucidate the differences in gut microbiota between Japanese patients with HSPC and CRPC and validate these differences in a transgenic mouse model. The results of this research are expected to contribute to our understanding of the role of gut microbiota in the progression of prostate cancer and the development of future therapeutic strategies.

2 | MATERIALS AND METHODS

2.1 | Study design

We analyzed the microbiota of patients with HSPC and CRPC. We collected rectal swab samples from 140 Japanese men with HSPC ($n=84$) or CRPC ($n=56$) from September 2020 through July 2022 at Kindai University Hospital, Osaka University Hospital, Osaka Police Hospital, Osaka General Medical Center, and Juntendo University Hospital. In the HSPC group, 23 patients who experienced biochemical recurrence following prostatectomy, external-beam radiation therapy (including intensity-modulated radiation therapy), or brachytherapy were included. Combined androgen blockade included ADT (in forms of luteinizing hormone-releasing hormone analog or antagonist) with antiandrogen such as bicalutamide or flutamide, or ARAT such as enzalutamide, abiraterone, apalutamide, or darolutamide. Regardless of the treatment method or treatment duration, HSPC and CRPC statuses were retrieved from the medical records of the enrolled patients at rectal swab sampling. The exclusion criteria were recent antibiotic use within 6 months of sample collection, inadequate sample, and data deficiency. In total, 136 samples were analyzed. This study was approved by the Institutional Review Board of Kindai University Hospital (IRB no. 13397-16), and written informed consent was obtained from all patients.

2.2 | Rectal swab collection and microbial DNA extraction

The samples were collected during a sterile digital rectal examination at urology clinics. The swabs were stored at −80°C until microbial DNA extraction. Microbial DNA was extracted from the samples using the DNeasy Power Soil Kit (Qiagen).

2.3 | Mouse experiments

Pten-deficient (PSA^{Cre}; $Pten^{loxP/loxP}$ on a C57BL/6J background) prostate-specific genetically engineered mice were bred in our laboratory as previously described. In these *Pten*-KO mice, prostatic tumors develop at 15 weeks of age, and when castration is performed at 20 weeks of age, castration resistance begins approximately 7 weeks postcastration.[12] *Pten*-KO mice were divided into three groups: Group A, which did not undergo surgical castration; Group

B, which was surgically castrated at 27 weeks of age; and Group C, which was surgically castrated at 23 weeks of age. Specifically, Group A represented intact CNPC, Group B represented HSPC at 3 weeks postcastration, and Group C represented CRPC at 7 weeks postcastration. Each group consisted of three mice, resulting in nine mice for analysis. Fecal samples were taken from the proximal and distal colon of mice at 30 weeks of age. Mice were housed at the Kindai University Faculty of Medicine Animal Facility according to institutional guidelines, and procedures were conducted in compliance with the standards for the use of laboratory animals. This study was approved by the Institutional Review Committee of Kindai University Faculty of Medicine (no. KDMS-18-008).

2.4 | Analysis of gut microbiota

Microbial DNA was analyzed using amplicon sequencing targeting the V1-V2 variable regions of the 16S rRNA gene. For the human samples, raw sequencing data were processed using the QIIME pipeline version 1.9.1 as the bioinformatics environment. A detailed analysis was carried out as previously described.[7] For the mouse samples, sequencing data were processed using CLC Genomics Workbench version 12.0 (Qiagen), and sequence reads were clustered into OTUs as previously described.[13] Operational taxonomic unit analysis was undertaken using the Microbiome Analyst 2.0 platform.[14]

2.5 | Statistical analysis

The Mann–Whitney *U*-test or Fisher's exact test was used to compare characteristics between the HSPC and CRPC groups. Alpha diversity was assessed by rarefaction analysis of evenness and richness, and we statistically compared each value at 10,000 sequences. Beta diversity was assessed by PCoA and ANOSIM. For comparison of each bacterial taxa, LEfSe was carried out using the Galaxy web application (https://huttenhower.sph.harvard.edu/galaxy/). p values <0.05 were considered statistically significant. Rarefaction analysis and PCoA were carried out using QIIME, ANOSIM was calculated using R version 4.0.2 package 'Vegan,' and the other statistical tests were undertaken using EZR software (https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).[15] Spearman's rank correlation coefficient analysis was used to evaluate the correlation of bacterial composition between groups of *Pten*-KO mice.

3 | RESULTS

3.1 | Patient demographics

We recruited 140 patients and, in the end, analyzed the gut microbiota in 136 patients (80 HSPC patients and 56 CRPC patients). The PSA levels at both diagnosis and rectal swab sampling were significantly higher in the CRPC group than in the HSPC group. Higher Gleason scores and T stage were observed in patients with CRPC, compared with those with HSPC. Although 23 patients (28.8%) were diagnosed with metastatic HSPC, the median PSA level at rectal swab collection was low, showing good response treatment

TABLE 1 Characteristics of Japanese men with hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC)

	CRPC ($n=56$)	HSPC ($n=80$)	p value
Age (years)	77 (72–82)	78 (73–82)	0.7000
Initial PSA (mg/dL)[a]	70.7 (22.2–157)	17.3 (7.22–86.4)	<0.001
PSA at rectal swab sampling (mg/dL)	1.59 (0.32–8.63)	0.01 (0.006–0.19)	<0.001
Gleason score			0.0017
6	3	9	-
7	3	22	-
8	24	23	-
≥9	26	26	-
BMI (kg/m^2)[b]	22.6 (20.1–24.4)	23.4 (21.2–24.9)	0.1600
T stage			<0.0010
T1	4	10	-
T2	8	41	-
T3	31	27	-
T4	11	2	-
Unknown	2	0	-
Metastases at diagnosis			0.0010
Yes	32	23	-
No	24	56	-
Unknown	0	2	-
Treatment method at rectal swab sampling			-
ADT only	3	5	-
CAB	11	61	-
Bicalutamide only	0	1	-
Abiraterone	4	12	-
Enzalutamide	13	0	-
Other ARAT	5	0	-
Docetaxel/ cabazitaxel	2	1	-
Ra-223	1	0	-
Drug-free	2	0	-
Unknown	14	0	-

Note: Values are presented as median (interquartile range) or number.

Abbreviations: ADT, androgen deprivation therapy; ARAT, androgen receptor axis-targeted therapy; BMI, body mass index; CAB, combined androgen blockade; PSA, prostate-specific antigen.

[a]There are two missing values of initial PSA in the HSPC group.

[b]There are five and two missing values of initial PSA in the HSPC and the CRPC groups, respectively.

such as ADT or CAB. Of the patients with HSPC, 82.2% received CAB, and bicalutamide was selected as the antiandrogen in most cases. The patient characteristics are summarized in Table 1.

3.2 | Taxonomic differences between HSPC and CRPC groups

The gut microbial composition of all samples at the phylum level is shown in Figure 1. Firmicutes and Bacteroides were dominant in most samples of the CRPC and HSPC groups (Firmicutes: median 62.5% in CRPC vs. 64.1% in HSPC, $p=0.65$; Bacteroides: median 26.8% in CRPC vs. 28.0% in HSPC, $p=0.57$). Alpha diversity was assessed using the Shannon index and Chao1 index. No significant difference was found between the CRPC and HSPC groups; however, the Shannon index tended to increase in the HSPC group ($p=0.082$; Figure 2A). Unweighted and weighted UniFrac PCoA showed no significant differences in the microbiota profiles between the CRPC and HSPC groups (Figure 2B). The LEfSe analysis, used to investigate differences in relative abundance of bacteria between the CRPC and HSPC groups, revealed that three taxa were significantly more abundant in the gut microbiota of the CRPC group, and eight taxa, including *Christensenella* and *Clostridium*, were significantly more abundant in the HSPC group ($p<0.05$, LDA score >|2.0|) (Figure 3). All three abundant taxa in the CRPC groups belonged to the Firmicutes phylum. *Gemella*, *Gemellales*, and *Lactobacillus* were significantly more abundant in the CRPC group ($p<0.05$).

3.3 | Mouse experiment

Using the *Pten*-KO mouse model, we evaluated the gut microbiota in the CNPC, HSPC, and CRPC groups (Figure 4A). Seven genera, including *Turicibacter*, *Clostridium*, *Lactococcus garvieae*, *Ruminococcus flavefaciens*, *Dorea*, *Lactobacillus*, and *Gemella*, were more abundant in CRPC compared with CNPC and HSPC (Figure 4B). These all belonged to the Firmicutes phylum. Among them, the proportions of *R. flavefaciens*, *Lactobacillus*, and *Gemella* were decreased in HSPC compared with CNPC. The increased abundance of *Gemella* and *Lactobacillus* in CRPC mice was consistent with the findings in Japanese patients.

4 | DISCUSSION

Recently, several reports have shown the characteristics of gut microbiota in patients with prostate cancer. We previously reported that in men who underwent prostatic needle biopsy, certain bacteria such as *Rikenellaceae*, *Alistipes*, and *Lachnospira*, were increased in the gut microbiota of patients with high-Gleason prostate cancer. Functional pathway analysis revealed that five metabolic pathways (starch and sucrose metabolism, phenylpropanoid biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, cyanoamino acid metabolism, and histidine metabolism) were positively associated with high-risk prostate cancer.[7] Significant compositional differences and greater abundance of *A. muciniphila* and Ruminococcaceae spp. were identified in the gut microbiota of men taking oral ATT, such as bicalutamide, enzalutamide, and abiraterone acetate, and in functional analyses, the pathways involved in steroid biosynthesis and steroid hormone biosynthesis were enriched in the oral ATT group.[8] *Akkermansia muciniphila* is found in abundance in CRPC patients receiving abiraterone acetate.[9] In contrast, in the gut microbiota of patients with mCRPC who received enzalutamide prior to pembrolizumab, *A. muciniphila* levels were reduced in responder samples.[10] An enrichment of the *Ruminococcus* and *Bacteroides* genera was observed in the mCRPC group compared with the HSPC group. Patients treated with enzalutamide but not abiraterone had a greater abundance of Ruminococcaceae, and pathway analysis showed that the steroid hormone biosynthesis pathway was enriched in the microbiota of

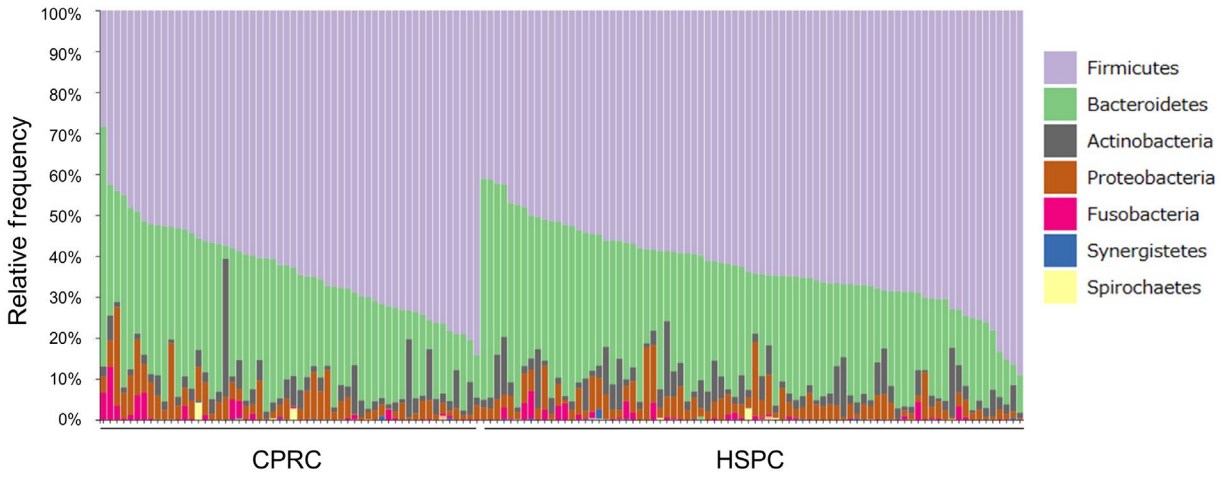

FIGURE 1 Composition of the gut microbiota at the phylum level in 136 samples from Japanese men with castration-resistant prostate cancer (CRPC) or hormone-sensitive prostate cancer (HSPC).

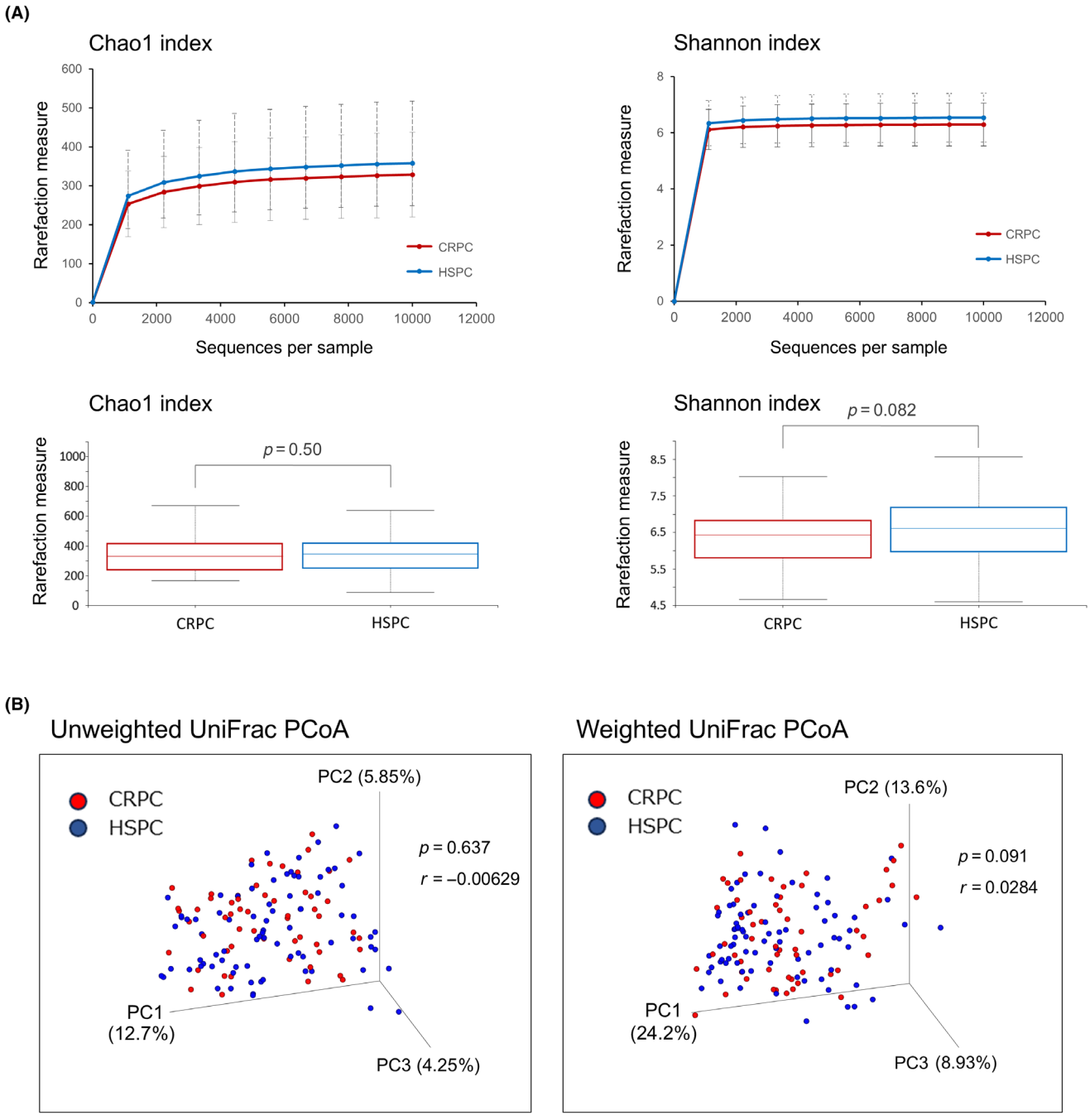

FIGURE 2 Diversity analysis of the gut microbiota in Japanese men with castration-resistant prostate cancer (CRPC) or hormone-sensitive prostate cancer (HSPC). (A) Rarefaction analysis rarefied to 10,000 sequences and boxplots of alpha diversity showing the Chao1 index (left) and Shannon index (right). Data are presented as the mean ± SD. Red and blue represent CRPC and HSPC, respectively. (B) Principal coordinate analysis (PCoA) plot of the unweighted (left) and weighted UniFrac distances (right), microbial composition. Red and blue dots represent CRPC and HSPC, respectively.

patients with mCRPC.[11] Thus, the gut microbiota can directly and indirectly affect treatment response to prostate cancer. A recent study has shown a significant difference in the gut microbiome between prostate cancer patients on ADT and prostatectomy and an enrichment of lipopolysaccharide and propanoate biosynthesis in the ADT groups through functional pathway analysis.[16]

The mechanism of a "gut–prostate axis," which involves the regulation of prostate cancer by the gut microbiota, has recently been elucidated. We discovered that antibiotic administration suppressed the growth of prostate cancer induced by HFD in transgenic mouse models of *Pten*-KO prostate cancer.[3] Antibiotics altered the composition of the gut microbiota, reducing SCFA-producing bacteria and subsequently suppressing IGF-1 expression. Additionally, HFD caused gut barrier dysfunction, leading to systemic inflammation resulting from the translocation of bacterial components into the systemic circulation. Furthermore, fecal

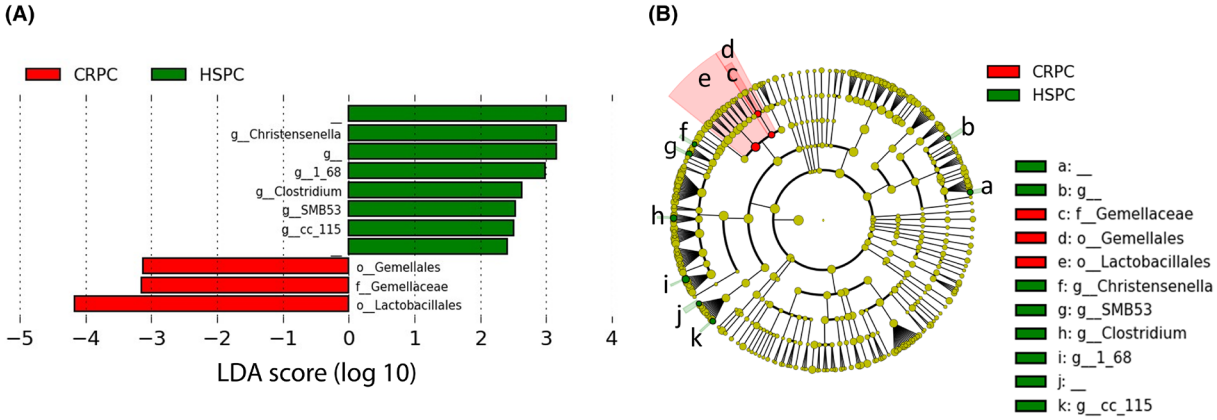

FIGURE 3 Linear discriminant analysis effect size (LEfSe) analysis between Japanese men with castration-resistant prostate cancer (CRPC) or hormone-sensitive prostate cancer (HSPC). (A) Histogram and (B) cladogram of linear discriminant analysis (LDA) scores showing the result of the LEfSe analysis, including significantly different operational taxonomic units (OTUs) among the groups ($p < 0.05$, LDA score >|2.0|). Red and green bars represent OTUs associated with the CRPC and HSPC groups, respectively.

microbiota transplantation from CRPC patients increased the abundance of *Ruminococcus*, which promoted prostate cancer progression. *Ruminococcus* is involved in androgen metabolism, which influences prostate cancer growth.[14] *Ruminococcus*, which is increased in patients with CRPC, can produce androgens, such as testosterone and dihydrotestosterone, from pregnenolone.[11] Notably, *Ruminococcus* is reported to be more abundant in prostate cancer patients, with dietary polyunsaturated long-chain fatty acids possibly regulating its levels.[17]

We previously reported that the abundance of Firmicutes was correlated with serum testosterone levels in Japanese men who had negative biopsy results.[18] Several studies have investigated the relationship between Firmicutes and androgen metabolism. For instance, *Clostridium*, a species of Firmicutes, can convert glucocorticoids into androgens, which can influence the host's androgen levels.[19] In this study, only bacteria belonging to the Firmicutes phylum were increased in patients with CRPC. This suggests that Firmicutes may increase in response to ADT in CRPC patients, potentially functioning as a source of androgen production in men with castrate levels of androgens.

Interestingly, the genus *Gemella* was found to be increased in elderly Japanese individuals with high testosterone levels.[18] The gut microbiota may undergo compositional changes to compensate for host deficiencies. It is possible that bacteria could respond to decreased testosterone levels in the host and promote androgen synthesis, potentially leading to the progression of CRPC. Our mouse model showed a correlation between *Ruminococcus* and *Gemella* and mouse CRPC. In the current Japanese cohort, *Ruminococcus* was not associated with CRPC. Based on the current data and our findings, bacteria associated with CRPC differs between Western cohorts and Japanese cancer patients. Given that *Ruminococcus* is involved in testosterone production in Western CRPC patients, it may be possible that *Gemella* and *Lactobacillus* contribute to androgen production in Japanese CPRC patients. Gut microbiota differs by race and geographical location. Moreover, genetic background and lifestyle factors, including diet, affect the gut microbiome. In a study identifying single nucleotide polymorphisms related to gut microbiota, five genetic regions were confirmed, but the results differed from those of Western populations. Genes related to dietary habits were also identified.[20,21] These differences in gut microbiota and genetics, which are also influenced by diet and race, may explain why the results in the Japanese population differ from those in other races.

There are several limitations in this study. In both the HSPC and CRPC populations, patient backgrounds were heterogeneous, encompassing cases such as post-radical prostatectomy, post-radiation therapy, and upfront ARAT, with wide variation in treatment durations. The sample size was relatively small. There were no direct comparisons with Western populations, and comparisons with other Asian populations were also lacking. We compared the gut microbiota between HSPC and CRPC patients and discussed its association with androgen metabolism. However, we did not have serum testosterone levels in this cohort. We also did not perform functional analyses to investigate the effects of *Gemella* and *Lactobacillus* on prostate cancer.

In conclusion, a comparative analysis of the gut microbiota between CRPC and HSPC in Japanese patients and mice revealed an increase in *Gemella* and *Lactobacillus*, suggesting a potential link between gut microbiota alterations and the development of castration resistance. These findings imply that gut microbiota could play a crucial role in the acquisition of castration resistance.

AUTHOR CONTRIBUTIONS

Koji Hatano: Conceptualization; writing – review and editing. **Eri Banno:** Writing – review and editing. **Daisuke Motooka:** Formal analysis; writing – review and editing. **Marco Antonio De Velasco:** Conceptualization; formal analysis; writing – review and editing. **Yurie Kura:** Data curation; investigation; writing – review and editing. **Shingo Toyoda:** Resources; writing – review and editing. **Mamoru Hashimoto:** Resources; writing – review and editing. **Shogo Adomi:**

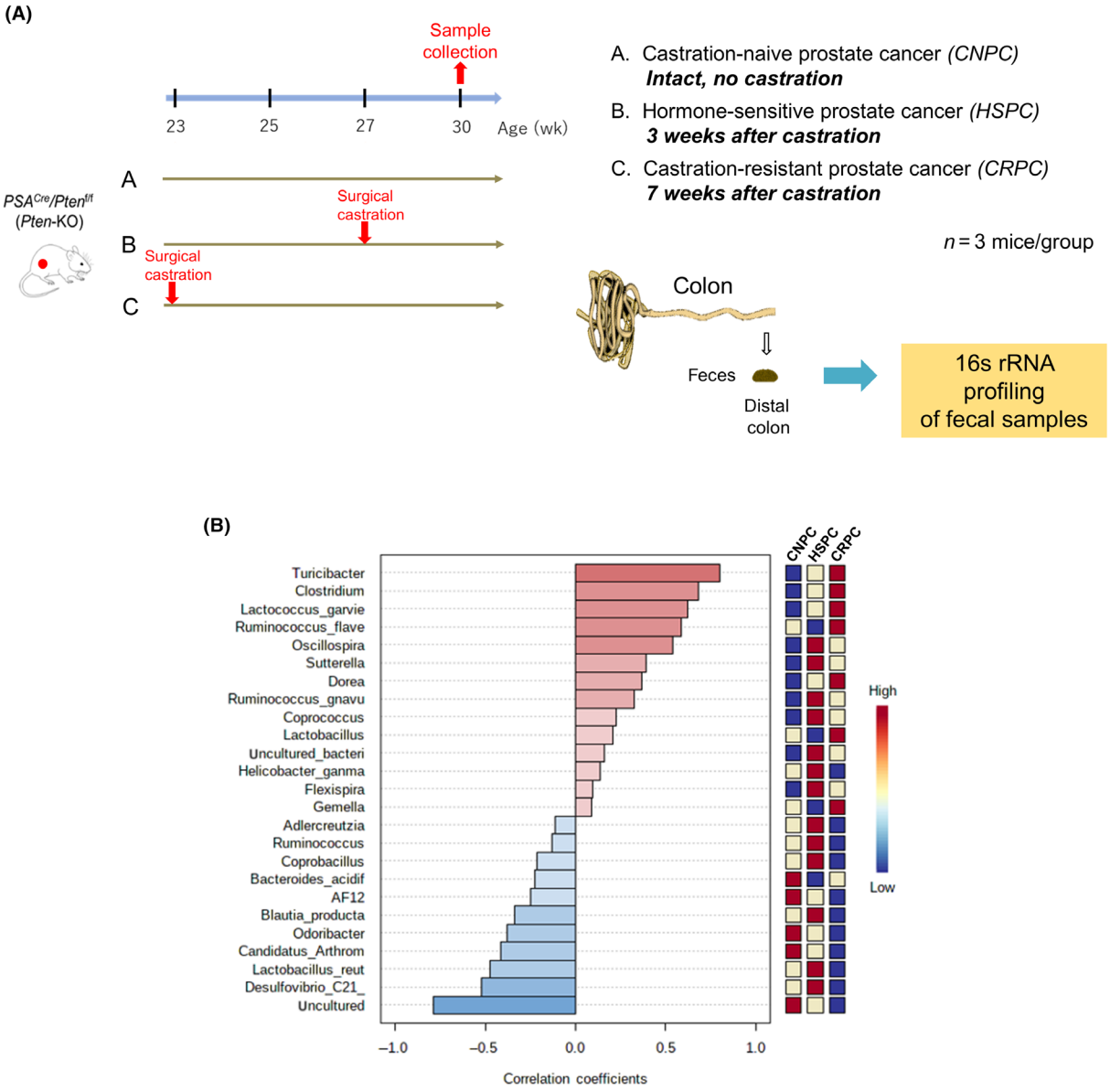

FIGURE 4 Top gut taxa correlated with response to androgen withdrawal in transgenic mice with prostate cancer. (A) Schematic representation of the experimental design. Prostate-specific *Pten*-conditional knockout (*Pten*-KO) mice were used to investigate the impact of androgen withdrawal by surgical castration on prostate cancer progression and gut microbiota composition. Mice were divided into three groups ($n = 3$ mice/group): Group A, noncastrated prostate cancer (CNPC); Group B, hormone-sensitive prostate cancer (HSPC); and Group C, castration-resistant prostate cancer (CRPC). Fecal samples were collected from the distal colon at 30 weeks (wk) of age for 16S rRNA profiling to analyze the gut microbiota composition. (B) Plot showing Spearman's correlation analysis shows the top 25 taxa. Blue represents negative correlations, red represents positive correlations. The deeper the color (blue or red), the stronger the correlation. The heatmap on the right side of the plot indicates the relative abundance according to group.

Resources; writing – review and editing. **Takafumi Minami:** Resources; writing – review and editing. **Kazuhiro Yoshimura:** Resources; writing – review and editing. **Toshiki Oka:** Data curation; investigation; writing – review and editing. **Junya Hata:** Resources; supervision. **Makoto Matsushita:** Formal analysis; supervision. **Tetsuya Takao:** Formal analysis; supervision. **Shingo Takada:** Resources; supervision. **Akira Tsujimura:** Resources; supervision. **Yoshiyuki Kojima:** Formal analysis. **Wataru Obara:** Formal analysis. **Shota Nakamura:** Formal analysis. **Hirotsugu Uemura:** Supervision. **Norio Nonomura:** Conceptualization; project administration. **Kazutoshi Fujita:** Conceptualization; project administration; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Nonomura Norio is an editorial board member of *Cancer Science*. The other authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Review Board: This study was approved by the Institutional Review Board of Kindai University Hospital (IRB no. 13397-16). The study was also approved by the Institutional Review Committee of Kindai University Faculty of Medicine (no. KDMS-18-008).

Informed Consent: Written informed consent was obtained from all recruited patients.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: Mice were housed at the Kindai University Faculty of Medicine Animal Facility according to institutional guidelines, and procedures were conducted in compliance with the standards for the use of laboratory animals.

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