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ORIGINAL RESEARCH—CLINICAL

# Decreased Abundance of Genus *Slackia* in Individuals With Obesity and Colorectal Adenoma

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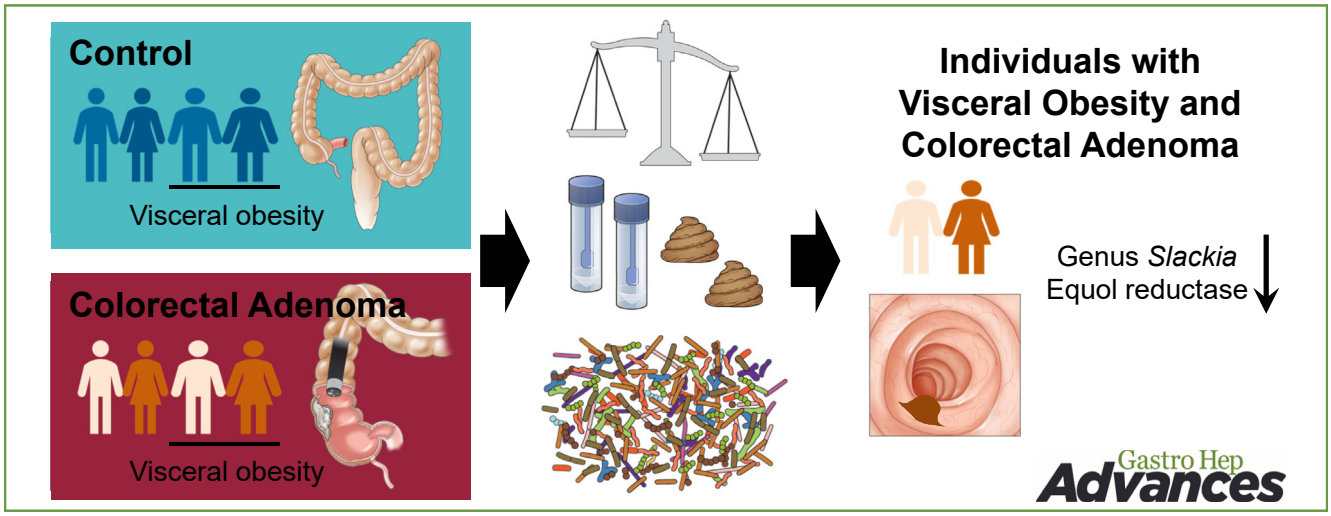


**BACKGROUND AND AIMS:** The increasing prevalence of obesity has significantly contributed to the global burden of colorectal cancer and the precancerous colorectal adenoma (CRA). Gut microbiota vary at each stage of colorectal carcinogenesis and participate in energy homeostasis. Elucidating gut microbiotal characteristics in obesity-related CRA may help prevent and treat colorectal tumors; however, this remains unclarified. Therefore, this study investigated the gut microbiota profile of patients with obesity-related CRA. **METHODS:** This hospital setting-based cross-sectional study included 113 participants (66 [without CRA control group] and 37 [with CRA group]; each group was divided into obese and nonobese groups) who underwent screening colonoscopy between June 2019 and January 2020. Gut microbiota were analyzed using 16S rRNA and polymerase chain reaction techniques and the data compared between the aforementioned groups. **RESULTS:** No between-group difference was observed in the diversity index; however, $\alpha$ diversity was the lowest in the obese CRA group. The CRA group had significantly higher and lower numbers of 26 and 17 genera, respectively. Genus *Slackia* was significantly lower in the obese CRA group than in the nonobese CRA group. Multivariate analysis of the quartiles according to genus *Slackia* relative abundance rates revealed that the first quartile was an independent risk factor for CRA (odds ratio, 3.57; 95% confidence interval 1.19–10.7). The proportion of equol reductase-positive participants was lowest in the obese CRA group ($P = .04$). Multivariate odds ratio for CRA was 5.46 (95% confidence interval 1.35–22.0) for genus *Slackia* and equol reductase-negative participants. **CONCLUSION:** Decreased abundance of genus *Slackia* and absence of equol reductase potentially influence obesity-related CRA development.

*Keywords:* Visceral Fat Accumulation; Precursor Lesion; Gut Microbiota; Fecal Bacteria

***Abbreviations used in this paper:*** **BMI, body mass index; CI, confidence interval; CRA, Colorectal adenoma; CRC, colorectal carcinoma; ddPCR, droplet digital polymerase chain reaction; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio; PCR, polymerase chain reaction; WC, waist circumference.**

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## Introduction

Colorectal cancer (CRC), the third most commonly diagnosed cancer, is a significant cause of cancer-related deaths.[1] Obesity, particularly visceral fat accumulation, and metabolic syndrome (MS) are significant predisposing factors for development of CRC[2] and its precursor lesion, colorectal adenoma (CRA).[3] Metabolic risk factors influenced the remarkable increase in cancer-related mortality between 2010 and 2019, contributing to a 35% increase in cancer-related deaths.[4] Various factors are associated with the development of obesity-related CRA,[5–7] namely, metabolic abnormalities, including insulin resistance and hyperinsulinemia, based on visceral fat accumulation,[8] altered adiponectin secretion,[9,10] and incretins,[11,12] chronic low-grade inflammation,[13,14] and decreased pancreatic enzyme levels.[15] However, the complex mechanisms underlying the reciprocity between obesity, MS, and CRA or CRC development remain unclarified.

Gut microbiota reportedly influence energy homeostasis significantly and directly impact the development of obesity and MS.[16] Furthermore, different gut microbiota and their metabolites are believed to be involved in each stage of CRC carcinogenesis, from CRA to advanced CRC.[17] For example, *Atopobium parvulum* increases only in patients with CRA and intramucosal CRC, whereas *Fusobacterium nucleatum* increases with CRC progression.[17] We had recently reported distinct alterations in the gut microbiota of patients with obesity and CRC, particularly a decrease in *Enterococcus faecalis*.[18] An in-depth understanding of the gut microbiotal characteristics in patients with CRC and CRA could enhance treatment and prevention strategies considerably. However, these characteristics remain unclear, particularly in patients with obesity-related CRA. Therefore, this study investigated the gut microbiota profile of patients with obesity-related CRA.

## Methods

### Study Design, Setting, and Participants

In this single-center cross-sectional study, asymptomatic adults who underwent a screening colonoscopy as part of a health checkup conducted between June 2019 and January 2020 ($n = 197$; Figure 1) were enrolled. A total of 111 individuals were considered eligible based on the following exclusion criteria: missing data ($n = 2$); unsuccessful total colonoscopy ($n = 1$); history of bowel resection ($n = 13$); history of gall bladder resection ($n = 2$); inflammatory bowel disease ($n = 2$); thyroid disease ($n = 1$); and the regular use of any medication ($n = 65$) including insulin, antidiabetics, antihyperlipidemics, antihypertensives, choleretics, antibiotics, and acid-suppressing agents. None of the participants were undergoing antiobesity treatment or had CRC. Eligible participants were classified into those with endoscopically diagnosed CRA (CRA group, $n = 37$) and undetectable CRA throughout the large intestine ($n = 74$). Among those with undetectable CRA, 8 with a history of endoscopic colorectal polyp resection were excluded to create a control group ($n = 66$). Participants in both groups were divided into obese (visceral obesity) and nonobese groups according to their waist circumference (WC). Visceral obesity was defined as a WC corresponding to a visceral fat area $\geq$100 $cm^2$ in Japanese individuals, namely $\geq$85 cm and $\geq$90 cm for men and women, respectively.[19]

### Clinical and Laboratory Assessments

Before colonoscopy, clinical information on smoking, alcohol consumption, and treatment history was obtained from all participants via a self-completed questionnaire. Current smoking was defined as smoking at least one cigarette/day within the past 12 months, and alcohol consumption, as >25 g/day of alcohol. Trained nurses obtained participants' anthropometric measurements, including height, weight, WC, and blood pressure. Body mass index was calculated as weight (kg) divided by the square height squared ($m^2$). The WC was measured at the umbilicus while the participants stood with their arms relaxed. Blood pressure was measured using an electronic sphygmomanometer.

Blood samples were collected from all participants after an overnight fast and before bowel preparation for colonoscopy, immediately separated into serum and plasma, and analyzed for high-density lipoprotein, low-density lipoprotein, triglyceride, hemoglobin A1c, and fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as follows: HOMA-IR = FPI ($\mu$IU/mL) $\times$ FPG (mmol/L)/22.5.

### Colonoscopy

All colonoscopies were performed using conventional video endoscopes (PCF-PQ260I, PCF-Q260AI, PCF-Q260AZI, or PCF-H290ZI; Olympus Medical Systems, Tokyo, Japan) after cleaning the bowel with a laxative (Laxoberon 0.75% solution, TEIJIN Pharma, Tokyo, Japan) and 1.5 L of polyethylene glycol electrolyte solution (MOVIPREP, EA Pharma Co, Japan). The endoscopists were blinded to the clinical and laboratory findings. Board-certified endoscopists from the Japanese Gastroenterological Endoscopy Society verified all the colonoscopy findings.

### Fecal DNA Extraction

Fecal samples were collected with a paper sheet (Raku-Ryu Cup Wide, Takahashi Keisei, Yamagata, Japan), specifically designed to prevent contamination and specimen loss, before bowel preparation for colonoscopy. Using a sterilized large brush kit (Techno Suruga Laboratory Co, Shizuoka, Japan), the collected samples were immediately placed in a tube containing guanidine thiocyanate solution. After vigorous mixing, samples were stored at $-80$ °C. Subsequently, 400 $\mu$L of the stored samples were vigorously vortexed in Easy bead tubes (AMR Inc, Gifu, Japan) at 2500 rpm for 3 min (Disruptor Genie, M&S Instruments Inc, Osaka, Japan). After being centrifuged at 100 $\times$g for 1 min, 200 $\mu$L of the supernatant was used to isolate fecal DNA using the KingFisher Duo Prime Purification System with MagMAX CORE Nucleic Acid Purification kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. The yield of the extracted DNA was evaluated using a Qubit HS Assay Kit (Thermo Fisher Scientific).

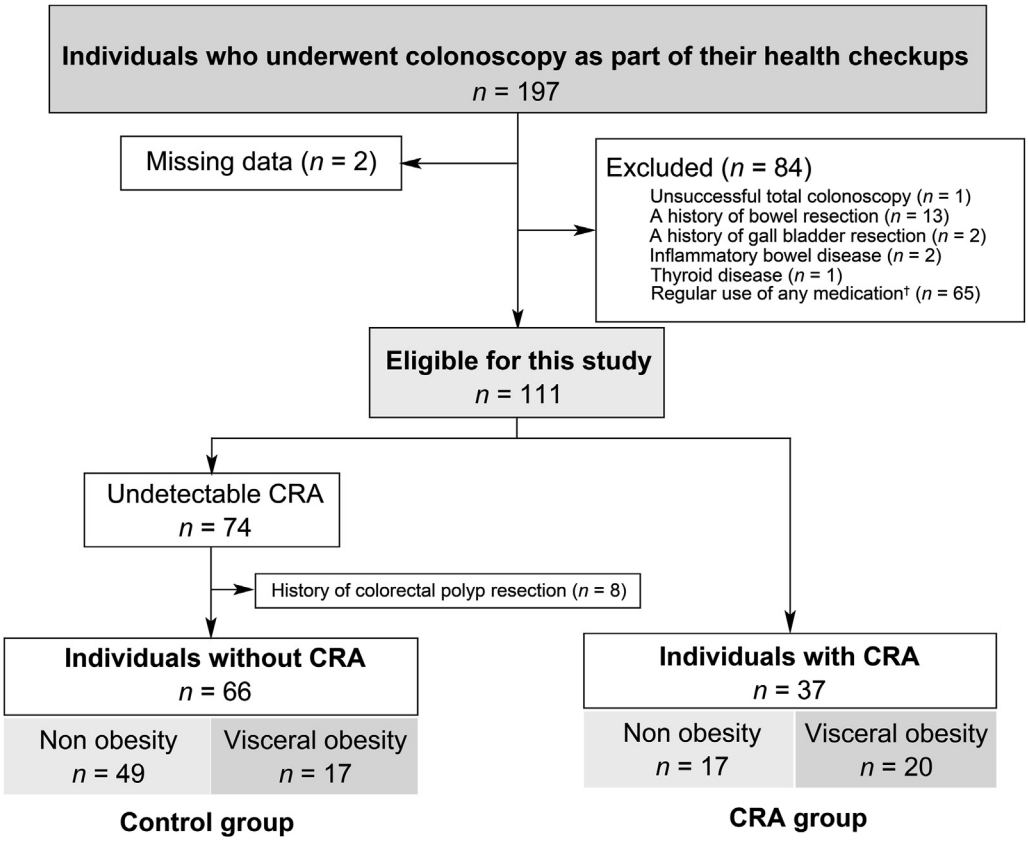



**Figure 1.** Flowchart of the participant selection process †Regular use of any drugs, including insulin, antidiabetics, antihyperlipidemics, and antihypertensives. CRA, colorectal adenoma.

## 16S rRNA Gene Sequencing

Multiplex polymerase chain reaction (PCR) was performed on the purified DNA samples using 2 primer pools corresponding to the V2, V4, and V8 (amplicon sizes of 250, 288, and 295 base pairs [bp], respectively) or the V3, V6–7, and V9 (amplicon sizes of 215, 260, and 209 bp, respectively) regions of the 16S rRNA (16S metagenomics kit, Thermo Fisher Scientific). Adapter sequences were added to the PCR products to obtain sequence libraries according to the procedure manual of the Ion AmpliSeq library kit (Thermo Fisher Scientific). To ensure the quality and size of each library, digital electrophoresis was performed using a D1000 ScreenTape on a 2200 TapeStation (Agilent Technologies, USA). Amplified libraries (30–140 pM) were subjected to emulsion PCR. After purification using biotin-coated beads, PCR products were loaded onto an Ion P1 chip v3. Ion semiconductor sequencing was performed using the Ion S5 (Thermo Fisher Scientific). To obtain taxonomy assignments, >40 bp read length in sequence files were run through the classification program using Kraken2 ver2.1.2 with indexes for more combinations of refSeq databases. The read depth against the 16S rRNA gene regions was calculated using the R statistic program version 4.0.4. Microbiome data that could confirm multiple reads in ±3 bp over the size of the amplicon against the V1–V9 regions using the In House R script were used to optimize mapping.

## Droplet Digital PCR for Genus Slackia Detection

Droplets were prepared by mixing the DNA template extracted from stool samples, genus *Slackia* specific primers[20] (forward 5′-GAC GGT ACC TGC AGA AGA AG-3′ and reverse 5′-CCC CGG CTT CGA CGG TGC CGC TT-3′), and droplet digital polymerase chain reaction (ddPCR) EvaGreen Supermix (Droplet Generator, Bio-Rad, CA, USA). These droplets were run in a C1000 Touch thermal cycler (Bio-Rad) for 5 min at 94 °C, followed by PCR with denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 30 s for 32 cycles. Subsequently, the fluorescence intensity of the droplets was measured using a QX200 Droplet Digital PCR System (Bio-Rad).

The PCR products obtained using genus *Slackia* specific primers were electrophoresed using a D1000 ScreenTape on a 2200 TapeStation (Agilent Technologies). A band was observed at 140 bp, consistent with the PCR product of DNA extracted from *Slackia equolifaciens* DSM 24851T strain (JCM16059, RIKEN BioResource Research Center, Tsukuba, Japan). Furthermore, the sequence of the PCR product was verified via Sanger sequencing and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis to confirm the specific identification of *S. equolifaciens*, *S. exigua*, *S. heliotrinireducens*, *S. isoflavoniconvertens*, and *S. piriformis*, which are present in the human gut microbiome.[20]

## Identification of Genes Involved in Equol Production

PCR was performed using the DNA template extracted from stool samples with primers specifically targeting the 3 following reductase genes essential for equol production: daidzein (*dzr*), dihydrodaidzein (*ddr*), and tetrahydrodaidzein (*tdr*) reductase. The following primers were used[21]: *dzr*, forward 5′-GAA GCT TGA

**Table 1.** Selected Characteristics of the Study Participants

| Participant characteristics | Control ($n = 66$) | CRA ($n = 37$) | *P* |
|---|---|---|---|
| Age, y | 53 (47–59) | 54 (47–59) | .44 |
| Sex, male | 42 (63.6) | 27 (72.9) | .33 |
| Current smoking | 3 (4.5) | 5 (13.5) | .13 |
| Alcohol consumption | 44 (66.6) | 31 (83.7) | .06 |
| Height, cm | 167.9 (159.7–172.4) | 169.0 (164.2–174.7) | .09 |
| Weight, kg | 67.1 (58.3–74.3) | 72.5 (63.9–86.8) | .02 |
| BMI[a], kg/m$^2$ | 23.6 (22.1–25.4) | 26.3 (22.1–28.5) | .06 |
| Waist circumference, cm | 81.4 (77.5–86.7) | 86.2 (80.0–94.0) | <.01 |
| Blood pressure, mmHg | | | |
| Systolic | 116 (108–124) | 120 (112–131) | .13 |
| Diastolic | 70 (64–78) | 77 (67–83) | .04 |
| HDL, mmol/L | 1.57 (1.27–1.86) | 1.52 (1.34–1.75) | .84 |
| LDL, mmol/L | 3.43 (2.85–3.99) | 3.41 (2.92–3.98) | .89 |
| TG, mmol/L | 1.07 (0.73–1.62) | 1.04 (0.83–1.64) | .39 |
| FPG, mmol/L | 5.10 (4.84–5.38) | 5.05 (4.94–5.38) | .74 |
| HbA1c, % | 5.7 (5.5–5.8) | 5.7 (5.6–6.0) | .16 |
| FPI, $\mu$U/mL | 4.1 (3.2–5.7) | 4.3 (3.5–6.8) | .38 |
| HOMA-IR[b] | 0.91 (0.66–1.42) | 0.94 (0.78–1.76) | .31 |

Values are expressed as median (IQR) or number (percentage). *P* values are evaluated using the Wilcoxon rank sum test, or $\chi^2$ test or Fisher's exact test.
BMI, body mass index; Control, participants without colorectal adenoma; CRA, participants with colorectal adenoma; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; LDL, low-density lipoprotein; TG, triglycerides.
[a]BMI, is calculated as weight in kg divided by height in m$^2$.
[b]HOMA-IR, is calculated as FPI ($\mu$U/mL) × FPG (mmol/L)/22.3.

TAT GGA CGA CT-3′ and reverse 5′-GGA ATA TGC ACC TGT TCC-3′; *ddr*, forward 5′-CTC GAY CTS GTS TAC AAC GT-3′ and reverse 5′-GAR TTG CAG ATK CCG AA-3′; and *tdr*: forward 5′-RTY AAC GGC RAY ATG CAG GT-3′ and reverse 5′-GGM AYY TCC ATG TTG TAG GA-3′. Electrophoresis using a 2200 TapeStation (Agilent Technologies) was considered positive if bands appeared at 204, 206, and 113 bp for *dzr*, *ddr*, and *tdr*, respectively.

### Statistical Analyses

Between-group comparisons were performed using the Wilcoxon rank-sum and $\chi^2$ tests and Fisher's exact test. Multiple comparisons were performed using the Kruskal–Wallis test for comparisons between 3 or more groups. Bray–Curtis, Shannon's, and Chao1 indices were calculated using the "vegan" 2.5–7 package in R (version 4.0.4). Spearman's rank correlation coefficient was used to correlate the proportion of each species in each group with the clinical data and ddPCR results. Logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for CRAs. Z-score calculations and statistical analyses were performed using the JMP Pro 17.0.0 (SAS Institute, Tokyo, Japan). $P < .05$ was considered statistically significant.

Further details of analysis in the Supplementary Methods.

## Results

### Selected Background Characteristics of the Study Participants

Median weight, WC, and diastolic blood pressure were significantly higher in the CRA group than in the control group (Table 1). No significant differences were observed between the CRA and control groups regarding median age, proportion of males, alcohol consumption, smoking, and median lipid, glucose, and insulin levels. In the control and CRA groups, participants with visceral obesity had metabolic abnormalities (Table 2). However, no significant differences were found in the background characteristics of these participants between the CRA and control groups.

### Gut Microbiotal Characteristics in Participants with Visceral Obesity and CRA

No significant differences were observed in the relative abundance rates of phyla (Figure A1A) and microbiota diversity between the CRA and control groups (Figure A2). Among participants with CRA and controls with or without visceral obesity, no significant differences were observed in the relative abundance rates of phyla (Figures A1B and A3), nonmetric multidimensional scaling (NMDS) plots based on Bray–Curtis distance matrices, observed species, and Chao1 and Shannon's indices, although $\alpha$ diversity metrics were low in the obese CRA group (Figure A2C–F). The relative abundance rates of 26 and 17 genera were significantly higher and lower, respectively, in the CRA group than in the control group (Figure 2A). Among these genera, the relative abundance of the genus *Slackia* was significantly lower in the obese CRA group than in the other groups, as evidenced by both 16S rRNA sequencing (Figure 2B) and ddPCR analysis (Figure 2C and D).

**Table 2.** Comparison of Characteristics Between the Nonobese and Obese Groups in Both the Control and CRA Groups

| Participant characteristics | Control | | | CRA | | | | |
|---|---|---|---|---|---|---|---|---|
| | Non-Ob ($n = 49$) | Ob ($n = 17$) | $P^c$ | Non-Ob ($n = 17$) | Ob ($n = 20$) | $P^d$ | $P^e$ | $P^f$ |
| Age, y | 54 (47–59) | 50 (46–56) | .09 | 56 (47–59) | 53 (50–57) | .46 | .14 | .29 |
| Sex, male | 27 (55.1) | 15 (88.2) | .01 | 10 (58.8) | 17 (85.0) | .13 | 1.00 | .01 |
| Current smoking | 2 (4.0) | 1 (5.8) | .75 | 2 (11.7) | 3 (15.0) | 1.00 | .60 | .43 |
| Alcohol consumption | 33 (67.3) | 11 (64.7) | .84 | 13 (76.4) | 18 (90.0) | .38 | .10 | .17 |
| Height, cm | 164.2 (158.2–171.5) | 171.2 (167.8–174.2) | <.01 | 168.3 (160.6–173.1) | 170.4 (165.5–175.3) | .24 | .92 | <.01 |
| Weight, kg | 63.7 (53.9–69.4) | 82.3 (73.3–87.5) | <.01 | 62.8 (56.2–67.1) | 86.2 (73.8–88.2) | <.01 | .37 | <.01 |
| BMI[a], kg/m$^2$ | 23.0 (21.5–24.3) | 27.6 (25.6–29.6) | <.01 | 21.9 (20.6–23.3) | 28.4 (26.6–30.0) | <.01 | .22 | <.01 |
| Waist circumference, cm | 79.5 (75.3–82.2) | 90.4 (88.7–97.9) | <.01 | 79.5 (76.0–83.2) | 93.7 (90.2–97.3) | <.01 | .19 | <.01 |
| Blood pressure, mmHg | | | | | | | | |
| Systolic | 114 (104–123) | 122 (114–126) | .02 | 117 (102–125) | 125 (118–137) | .03 | .41 | .01 |
| Diastolic | 67 (61–75) | 76 (72–85) | <.01 | 70 (64–81) | 81 (72–84) | .01 | .61 | <.01 |
| HDL, mmol/L | 1.75 (1.39–1.91) | 1.26 (1.11–1.47) | <.01 | 1.65 (1.39–1.91) | 1.43 (1.28–1.65) | .10 | .14 | <.01 |
| LDL, mmol/L | 3.38 (2.81–3.93) | 3.77 (3.25–4.13) | .15 | 3.15 (2.84–3.85) | 3.47 (2.94–4.01) | .38 | .57 | .40 |
| TG, mmol/L | 0.85 (0.69–1.38) | 1.45 (1.37–2.02) | <.01 | 0.97 (0.77–1.38) | 1.35 (0.89–1.89) | .04 | .50 | <.01 |
| FPG, mmol/L | 5.16 (4.82–5.38) | 5.10 (4.88–5.38) | .95 | 5.05 (4.94–5.16) | 5.19 (4.91–5.55) | .27 | .53 | .74 |
| HbA1c, % | 5.6 (5.5–5.8) | 5.8 (5.4–6.0) | .30 | 5.7 (5.5–6.0) | 5.7 (5.5–6.0) | .52 | .55 | .33 |
| FPI, $\mu$U/mL | 3.9 (2.8–4.7) | 7.1 (4.3–9.8) | <.01 | 3.6 (2.6–3.9) | 6.5 (4.8–8.5) | <.01 | .65 | <.01 |
| HOMA-IR[b] | 0.87 (0.64–1.12) | 1.72 (0.90–2.40) | <.01 | 0.79 (0.57–0.92) | 1.60 (0.97–1.99) | <.01 | .78 | <.01 |

Values are expressed as median (IQR) or number (percentage).
BMI, body mass index; Control, participants without colorectal adenoma; CRA, participants with colorectal adenoma; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; LDL, low-density lipoprotein; ob, visceral obese; TG, triglyceride.
[a]BMI, is calculated as weight in kg divided by height in m$^2$.
[b]HOMA-IR, is calculated as FPI ($\mu$U/mL) × FPG (mmol/L)/22.3. *P* values are evaluated using the Wilcoxon rank sum test or $\chi^2$ test.
[c]Non-Ob vs Ob in the control group.
[d]Non-Ob vs Ob in the CRA, group.
[e]Ob in the control vs Ob in the CRA, group.
[f]Multiple comparisons are performed with the Kruskal–Wallis test or $\chi^2$ test.

### Association Between CRA and Genus Slackia

After dividing the participants into quartiles according to genus *Slackia* relative abundance rates, those in the first quartile with the lowest rate were rated the highest in the obese CRA group (Table A1). No clinical characteristics were associated with these quartiles (Table A2). Univariate analysis using logistic regression revealed significantly high ORs for CRA regarding weight, body mass index, WC, and visceral fat accumulation (Table 3). The OR for CRA was more in the first quartile and tended to increase as the quartile decreased. Multivariate analyses, including age, male sex, current smoking, alcohol consumption, visceral obesity, and diastolic blood pressure, revealed consistent trends (Table 3). Compared with the other quartiles, the first quartile was an independent risk factor for CRA (3.57, 95% CI 1.19–10.7, $P = .02$).

### Equol-Producing Reductase Genes Between the CRA and Control Groups

*Slackia* spp. are equol-producing bacteria.[21] Using fecal DNA, the proportion of equol reductase gene-positive participants, in whom any of the reductases (*ddr*, *tdr*, or *dzr*) were detected, was the lowest in the obese CRA group (Figure 3, Table A3). Compared with participants who were genus *Slackia* and equol reductase-positive, the OR for CRA on multivariate analysis was 5.46 (95% CI 1.35–22.0) for participants who were genus *Slackia* and equol reductase-negative. Odds were higher for genus *Slackia*-negative participants than for equol reductase-negative participants (Figure 3).

## Discussion

This study demonstrated that individuals with visceral obesity and CRA had a low abundance of genus *Slackia* in their stool. *Slackia* is a genus of the phylum Actinobacteria, and 6 species have been isolated from human feces: *S. heliotrinireducens*,[22] *S. exigua*,[22] *S. faecicanis*,[23] *S. isoflavoniconvertens*,[24] *S. equolifaciens*,[25] and *S. piriformis*.[26] Although details regarding the average abundance of genus *Slackia* in the healthy human gut microbiota are limited, its decreased abundance has been reported in several diseases. Patients with cirrhosis with excess extracellular fluid, measured using multifrequency

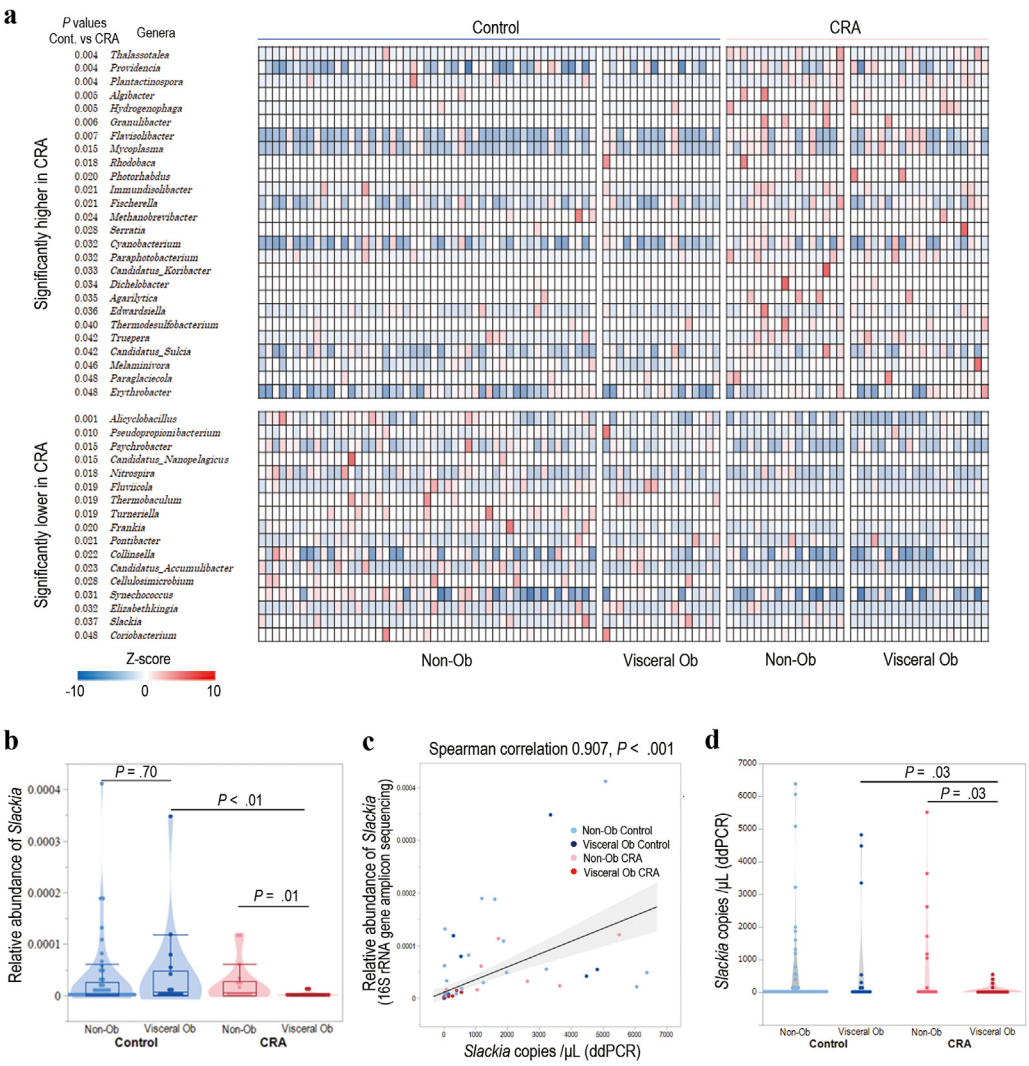



**Figure 2.** Heatmap illustrating the Z-score distribution of genera in the CRA and control groups (A) In the obese CRA group, the genus *Slackia* was significantly lower than that in the obese control group or nonobese CRA group (B) The abundance of genus *Slackia* determined via 16S rRNA sequencing and ddPCR was well-correlated (C) the copy number of genus *Slackia* examined by ddPCR was significantly lower in participants with visceral obesity and CRA (D). Control, participants without colorectal adenoma; CRA, participants with colorectal adenoma; ddPCR, droplet digital polymerase chain reaction; Ob, obese.

bioelectrical impedance analysis, had decreased abundance of fecal genus *Slackia* compared with patients with cirrhosis and without excess extracellular fluid.[27] Patients with polycystic ovarian syndrome, who are at risk of obesity, insulin resistance, and metabolic abnormalities, have a decreased abundance of genus *Slackia* compared with healthy individuals.[28] *Slackia* spp. was not detected in the gut microbiota of patients with active Vogt-Koyanagi-Harada disease compared with that in patients with noninfectious anterior scleritis or healthy individuals.[29] The aforementioned study discussed the potential of decreased abundance of *Slackia* spp. in affecting the pathogenesis of Vogt-Koyanagi-Harada disease in association with elevated proinflammatory cytokines such as tumor necrosis factor-$\alpha$, interleukin (IL)-1, and IL-6. We had previously reported that individuals with obesity and CRA have a chronic systemic low-grade inflammatory state and that elevated serum IL-6 levels are an independent risk factor for CRA.[13] Decreased abundance of genus *Slackia* may contribute to an increased risk of CRA due to chronic systemic low-grade inflammation in individuals with obesity.

Certain species such as *S. isoflavoniconvertens*, *S. equolifaciens*, and *Slackia* sp. strain NATTS, metabolize plant isoflavones such as daidzein and genistein in the gut, resulting in equol production.[21,25,30,31] Approximately 30% of Europeans and Americans and 50% of Japanese are capable of producing equol.[32,33] Accordingly, the overall equol reductase positivity rate in the present study was 51.4% (53/103, Table A3). Equol has estrogen-like,[34,35] antiandrogenic,[36] and antioxidant effects.[37] Moreover, equol has demonstrated anti breast cancer properties[38] and reportedly inhibits the growth of prostate cancer cells by degrading the androgen receptor via the S-phase kinase-associated protein 2.[39] Polyphenols, including equol,

**Table 3.** Odds Ratio for Colorectal Adenoma

| Characteristics | *n* | Univariate OR (95% CI) | *P* | Multivariate 1 OR (95% CI) | *P* | Multivariate 2 OR (95% CI) | *P* |
|---|---|---|---|---|---|---|---|
| Age, y | | 1.03 (0.96–1.11) | .30 | 1.06 (0.97–1.15) | .14 | 1.05 (0.98–1.15) | .14 |
| Sex, male | | 1.54 (0.63–3.72) | .33 | 0.64 (0.20–2.07) | .46 | 0.67 (0.21–2.09) | .50 |
| Current smoking | | 3.28 (0.73–14.6) | .11 | 3.53 (0.58–21.2) | .16 | 3.49 (0.58–20.9) | .17 |
| Alcohol consumption | | 2.58 (0.93–7.11) | .06 | 1.91 (0.61–5.94) | .26 | 1.95 (0.63–6.02) | .24 |
| Height, cm | | 1.49 (0.99–1.10) | .08 | | | | |
| Weight, kg | | 1.04 (1.00–1.07) | .01 | | | | |
| BMI[a], kg/m$^2$ | | 1.12 (1.00–1.26) | .03 | | | | |
| Waist circumference, cm | | 1.06 (1.01–1.11) | <.01 | | | | |
| Visceral obesity[b] | | 3.39 (1.44–7.93) | <.01 | 3.32 (1.10–10.0) | .03 | 3.16 (1.13–8.79) | .02 |
| Blood pressure, mmHg | | | | | | | |
| Systolic | | 1.02 (0.99–1.05) | .11 | | | | |
| Diastolic | | 1.03 (0.99–1.07) | .05 | 1.02 (0.97–1.07) | .27 | 1.02 (0.97–1.08) | .26 |
| HDL, mmol/L | | 0.99 (0.97–1.07) | .68 | | | | |
| LDL, mmol/L | | 1.00 (0.98–1.01) | .79 | | | | |
| TG, mmol/L | | 1.00 (0.99–1.00) | .34 | | | | |
| FPG, mmol/L | | 1.00 (0.96–1.04) | .95 | | | | |
| HbA1c, % | | 1.32 (0.52–3.33) | .55 | | | | |
| FPI, $\mu$U/mL | | 1.08 (0.94–1.24) | .25 | | | | |
| HOMA-IR[c] | | 1.36 (0.76–2.44) | .29 | | | | |
| Quartiles of *Slackia* spp. abundance rate, $\times 10^{-6}$ | | | | | | | |
| Quartile 1, <0.57 | 25 | 2.94 (0.91–9.46) | .07 | 3.14 (0.81–12.1) | .09 | | |
| Quartile 2, 0.57–<3.57 | 27 | 1.59 (0.49–5.12) | .43 | 0.89 (0.22–3.47) | .86 | | |
| Quartile 3, 3.57–<16.0 | 25 | 1.05 (0.30–3.61) | .93 | 0.75 (0.18–3.06) | .69 | | |
| Quartile 4, ≥16.0 | 26 | 1.00 (reference) | | 1.00 (reference) | | | |
| *P* For trend | | | .04 | | .08 | | |
| Q1 vs Q2–Q4 | | 2.43 (0.97–6.11) | .05 | | | 3.57 (1.19–10.7) | .02 |

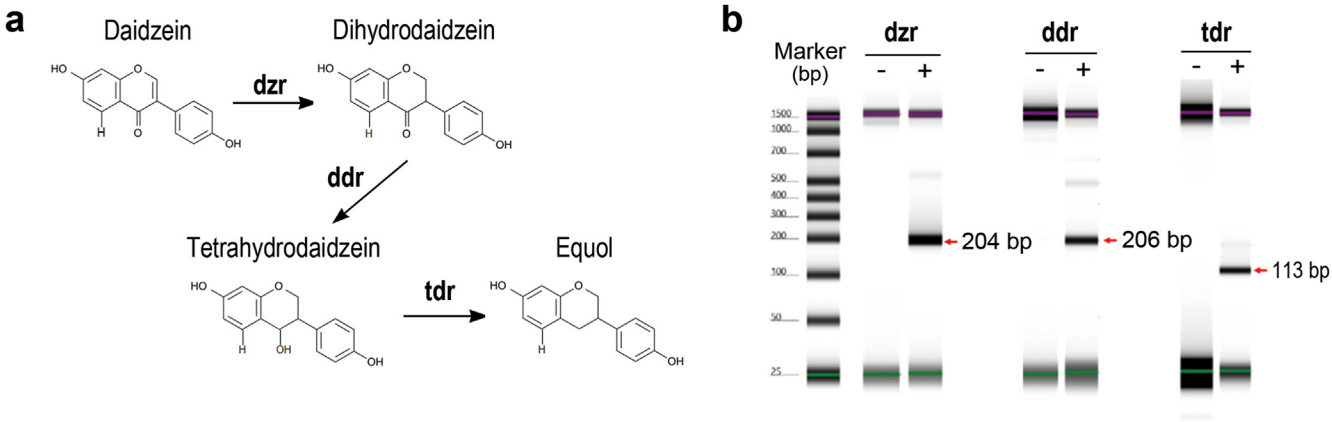
Values are expressed as odds ratios (95% CI).

BMI, body mass index; CI, confidence interval; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; n, number; OR, odds ratio; TG, triglyceride; WC, waist circumference.

[a]BMI, is calculated as weight in kg divided by height in m$^2$.

[b]Visceral obesity is defined as WC, corresponding to a visceral fat area of ≥100 cm$^2$ in Japanese individuals, namely, ≥85 cm for men and ≥90 cm for women.[19]

[c]HOMA-IR, is calculated as FPI ($\mu$U/mL) × FPG (mmol/L)/22.3.




c

| *n* Control/ CRA | | Multivariate | | | Odds ratio (95% CI) *P* value |
|---|---|---|---|---|---|
| 7/ 9 | Univariate | ***Slackia* -, Equol-** | 1.27 (0.18–8.93) 0.80 | 2.82 (0.68–11.6) 0.14 | 5.46 (1.35–22.0) 0.01 |
| 4/ 4 | | 1.28 (0.22–7.34) 0.77 | ***Slackia* -, Equol+** | 2.21 (0.37–13.1) 0.38 | 4.27 (0.72–25.1) 0.10 |
| 22/ 12 | | 2.35 (0.70–8.20) 0.16 | 1.83 (0.37–9.05) 0.44 | ***Slackia* +, Equol-** | 1.93 (0.65–5.66) 0.22 |
| 33/ 12 | | 3.53 (1.09–12.0) 0.03 | 2.75 (0.57–13.3) 0.20 | 1.50 (0.56–3.97) 0.41 | ***Slackia* +, Equol+** |

**Figure 3.** Daidzein conversion pathway by reductases (A) Representative images of PCR bands for 3 reductase genes. (B) Odds ratios for CRA according to genus *Slackia* abundance and equol reductase genes in stool (C). Multivariate analyses included age, male sex, current smoking status, alcohol consumption, visceral obesity, and diastolic blood pressure. The upper row represents the odds ratio; middle row, the 95% CI; and lower row, the *P* value. Equol+ was defined as positivity for any of the 3 reductases. Based on the vertical group, the odds ratio of the horizontal group was calculated using logistic regression analysis. bp, base pair; CI, confidence interval; control, participants without colorectal adenoma; CRA, participants with colorectal adenoma; *ddr*, dihydrodaidzein reductase; *dzr*, daidzein reductase; PCR, polymerase chain reaction; *tdr*, tetrahydrodaidzein reductase.

induce DNA damage and apoptosis in CRC cells by inhibiting bromodomain-containing protein 9.[40] A decrease in dietary isoflavone intake can increase the risk of CRA, implying the involvement of the estrogen-like effects of plant isoflavones.[41] In the human colon, estrogen receptor $\beta$, which has a high affinity for phytoestrogens, is predominant and reportedly inhibits the growth of colorectal tumors.[41] Therefore, equol produced by *Slackia* spp. may inhibit CRA development.

Other equol-producing bacteria[42] could inhibit CRA development, especially in genus *Slackia*-negative and equol reductase-positive individuals. Furthermore, the higher OR for CRA observed in genus *Slackia*-negative and equol reductase-positive participants than in genus *Slackia*-positive and equol reductase-negative participants suggests that *Slackia* spp. may possess a unique inhibitory mechanism for CRA that is independent of the effects of equol. However, no published literature has elucidated the effects of *Slackia* spp. on the colonic mucosa and tumors, aside from its influence by equol. This highlights the complexity of gut microbiota and their metabolic interactions and the need for further research to comprehend their roles in CRA development.

Excluding the genus *Slackia*, the relative abundance rates of 26 and 16 genera were significantly higher and lower, respectively, in the CRA group than in the control group in this study. Among these, 6 bacterial genera have previously been reported to be associated with either CRA or CRC (Table A4). However, none of the other genera have been identified in association with CRA and CRC. These genera may be potential new factors involved in CRA

development. Therefore, further studies, including a large number of patients with CRA, are required to clarify this association.

This study had some limitations. First, its case-control design limits the establishment of a causal relationship between decreased abundance of genus *Slackia* and equol reductase level and CRA development. Therefore, basic experiments and prospective studies are necessary to establish these causal relationships. Second, because of the small number of cases in this study, the generalizability of the findings to larger populations, other regions, and ethnic groups may not be possible. Third, the sensitivity of fecal equol reductase detection via PCR is low[21]; therefore, considering individuals as reductase-positive if they test positive for any of the 3 reductases was a reasonable approach in this study. However, more accurate identification of reductase-positive individuals and the correlation of reductase with actual fecal equol concentrations requires further investigation. Fourth, the existence of data on the consumption of soybean products in each group may support our findings; however, a dietary intake survey was not conducted. Fifth, to understand the factors contributing to decreased abundance of genus *Slackia* and their impact on homeostasis, factors such as diet, exercise habits, and physical activity that affect the gut microbiota, and gut metabolites need detailed analysis. Therefore, future studies should examine patients with early-stage CRC to determine whether these alterations are specific to CRA.

In conclusion, this study is the first to demonstrate that decreased abundance of genus *Slackia* and absence of equol reductase in the stool of patients with visceral obesity and CRA. These findings indicate that genus *Slackia* and equol reductase are associated with an increased risk of CRA and may influence obesity-related CRA development. Furthermore, the findings provide valuable insights into the role of gut microbiota in obesity-related CRA development and indicate that modulation of these factors could help potentially prevent and treat CRA.

## Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2024.02.010.

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**Conflicts of Interest:**
The authors disclose no conflicts.

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**Ethical Statement:**
This study was approved by the Ethics Committee of Yamagata University Faculty of Medicine (#2018-260) and Tohoku Central Hospital (#807-6). Written informed consent was obtained from all participants prior to study commencement.

**Data Transparency Statement:**
The data that support the findings of this study will be made available to other researchers upon reasonable request and approval from the corresponding author. Nucleotide sequence data reported are available in the DDBJ Sequenced Read Archive under the accession numbers DRA017281.

**Reporting Guidelines:**
Helsinki Declaration.