Association of *Escherichia coli* containing polyketide synthase in the gut microbiota with colorectal neoplasia in Japan

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**Abstract**

*Escherichia coli* containing polyketide synthase in the gut microbiota (*pks*⁺ *E coli*) produce a polyketide-peptide genotoxin, colibactin, and are suspected to play a role in the development of colorectal neoplasia. To clarify the role of *pks*⁺ *E coli* in the early stage of tumorigenesis, we investigated whether the *pks* status of *E coli* was associated with the prevalence of colorectal neoplasia. This cross-sectional analysis of data from a prospective cohort in Izu Oshima, Japan included asymptomatic residents aged 40-79 years who underwent screening colonoscopy and provided a stool sample. We identified 543 participants with colorectal neoplasia (22 colorectal cancer and 521 adenoma) as cases and 425 participants with normal colon as controls. The *pks* status of *E coli* was assayed using stool DNA and specific primers that detected *pks*⁺ *E coli*. The proportion of *pks*⁺ *E coli* was 32.6% among cases and 30.8% among controls. Compared with those with *pks*⁻ *E coli*, the odds ratio (OR) (95% confidence interval) for participants with *pks*⁺ *E coli* was 1.04 (0.77-1.41) after adjusting for potential confounders. No statistically significant associations were observed regardless of tumor site or number of colorectal adenoma lesions. However, stratified analyses revealed increased ORs among participants who consumed cereals over the median intake or vegetables under the median intake. Overall, we found no statistically significant
1 | INTRODUCTION

Colorectal cancer is the third most common cancer worldwide. 1 The majority of cases are sporadic and arise through the traditional adenoma-carcinoma pathway. 2 Accumulating epidemiological evidence indicates the important role of lifestyle and environmental factors in the development of colorectal neoplasia but its etiology is not fully understood. 2,3

Recently, attention has focused on a potential role of the gut microbiota in colorectal carcinogenesis. 4,5 Escherichia coli from the B2 phylogenetic group possesses a genomic island named polyketide synthetase (pks), which is thought to produce a polyketide-peptide genotoxin, colibactin. E coli containing pks (pks+ E coli) has been shown to induce DNA double-strand breaks, cell cycle arrest, mutations, and chromosomal instability in eukaryotic cells. 6-9 Colibactin also alkylates DNA in vivo and DNA adducts have been identified in mammalian cells and mice exposed to pks+ E coli. 10 Five studies have compared the prevalence of pks+ E coli between patients with and without colorectal neoplasia but findings are inconsistent: 11-13 three found significantly higher prevalence among colorectal cancer cases than the control group, 11-13 whereas two showed no statistically significant difference. 14,15 Two of these studies examined the prevalence of colorectal adenoma and observed no statistically significant difference, although one showed a higher prevalence of colorectal adenoma cases. 13,14 However, these studies included a relatively small number of colorectal neoplasia cases and did not adjust for potential confounders.

Here, to better understand the role of pks+ E coli in the early stage of the adenoma-carcinoma sequence, we investigated whether the pks status of E coli was associated with the prevalence of colorectal neoplasia. The study was carried out under a cross-sectional design using data from a prospective cohort in Izu Oshima, Japan, which included 543 cases (22 colorectal cancer and 521 adenoma cases) and 425 controls. We also tested the hypothesis that lifestyle and dietary factors modify the association between pks+ E coli and the prevalence of colorectal neoplasia.

2 | MATERIALS AND METHODS

2.1 | Study cohort

The Oshima study was carried out under a prospective cohort design in Izu Oshima, a small island near the mainland Japanese island of Honshu. The study aimed to evaluate the diagnostic ability and effectiveness of colorectal cancer screening techniques and biomarkers. 16 We recruited all island residents aged 40-79 years without uncontrolled complications, including unstable angina, acute myocardial infarction, heart failure, chronic respiratory disease, and bleeding tendency, which would hinder the safe performance of colonoscopy. The baseline survey, including a self-administered questionnaire survey, blood and stool sample collection, 2-day fecal immunochemical test, and screening colonoscopy, was undertaken between November 2015 and June 2017. Of 4645 residents, 1367 provided written informed consent. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

2.2 | Questionnaire survey

All participants were asked to complete a self-administered questionnaire before the screening colonoscopy. The questionnaire enquired about lifestyle factors, such as personal medical history, present medication, family history of cancer, cigarette smoking, alcohol drinking, and physical activity, among others. It also included a food frequency questionnaire (FFQ). The FFQ was originally used in the Japan Public Health Center-based Prospective Study for the Next Generation (JPHC-NEXT Study), and contained an added item, kusaya, a dried fish that is popular in Izu Oshima. The original FFQ was validated in middle-aged and elderly Japanese using 12-day weighted food records (3 days per season). 17 It consists of 67 food and beverage items with nine frequency categories and standard portions/units, and asks about the usual consumption of listed foods during the previous year. Frequency response choices for food items are less than once per month, 1-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, once per day, 2-3 times per day, 4-6 times per day, and 7 or more times per day. Standard portion sizes are specified for each food item in the three “amount” choices of small (50% smaller than standard), medium (standard), and large (50% larger). Daily food intake is calculated by multiplying frequency by standard portion and relative size for each food item. Intake of energy and nutrients is calculated using the Standard Tables of Food Composition in Japan 2015. 18

2.3 | Stool sample collection and laboratory analysis

Stool sample collection vials containing 3 ml GuSCN solution (TechnoSuruga Laboratory Co., Ltd) along with information about
the collection procedure were sent to participants. The sample was collected by the participant prior to preparation for the colonoscopy procedure and stored at room temperature until the colonoscopy procedure. The vials were then stored at −80°C until analysis. Stool DNA was extracted from a portion of frozen stool by the bead beating method, as detailed elsewhere.19

To confirm that the E coli was a pks+ strain, PCR was carried out to amplify genes from the clb cluster using bacterial genomic DNA as a template. The details have been reported elsewhere.20,21 In brief, two primer sets were used to amplify each of the genes in the cluster, namely clbB-F/clbB-R for clbB and clbQ-F/clbQ-R for clbQ. Participants for whom clbB and clbQ were unambiguously detected from feces were defined as pks+E coli individuals.

2.4 | Colonoscopy procedure

All colonoscopy procedures were undertaken to examine the whole colon and rectum using video colonoscopes with a magnification function (CF-HQ290ZI, PCF-Q260AZI; Olympus Co.). A total of 25 experienced endoscopists who were board-certified by the Japanese Gastrointestinal Endoscopy Society participated in the study and carried out the colonoscopies. Polyethylene glycol or magnesium citrate solution was given in the morning of the day of the procedure for bowel preparation.

2.5 | Selection of cases and control

Of the 1367 participants, we excluded participants who did not undergo colonoscopy, underwent incomplete examination (cecum not reached in colonoscopy), had a history of cancer, colorectal polyp, colorectal surgery, or colonoscopic treatment based on a self-administered questionnaire, or who did not provide a stool sample. We further excluded participants who reported extreme energy intakes (below the 2.5 or over the 97.5 percentiles), leading 1034 participants. Among these, 22 participants had colorectal cancer, 521 had one or more adenomas, 49 had hyperplastic polyp only, and 17 had other lesions (eg, neuroendocrine tumor, nonneoplastic lesion) based on a pathologically confirmed diagnosis. The remaining 425 had a normal colon. After exclusion of participants with hyperplastic polyp only or other lesions, we considered the 543 participants with colorectal neoplasia (colorectal cancer or adenoma) as cases and the 425 participants with normal colon as controls. Additionally, we defined advanced colorectal neoplasia as comprising colorectal cancer and advanced adenoma (adenoma with a diameter of 10 mm or more, high-grade dysplasia, or prominent villous component).22,23 Subsites of colon neoplasia were defined by a location in the proximal colon (cecum and ascending and transverse colon) or distal colon (descending and sigmoid colon).

2.6 | Statistical analysis

Dietary intakes of food groups and nutrients were energy-adjusted by the residual regression method. Case-control comparisons for mean, median, and proportions were tested with the t test, Wilcoxon rank-sum test, and χ² test, respectively. An unconditional logistic regression model was used to estimate odds ratio (OR) and 95% confidence intervals (CI) of the prevalence of colorectal neoplasia according to the pks status of E coli. The regression models were adjusted for age (continuous), sex, cigarette smoking (never smokers, past smokers, and <20, 20–39, ≥40 pack-years for current smokers), alcohol consumption (nondrinkers, past drinkers, occasional drinkers, and <150, 150–299, 300–449, >450 g/wk for regular drinkers), body mass index (kg/m²) (<21, 21–23.9, 24–26.9, 27–29.9, ≥30), physical activity (metabolic equivalent-h/d, quartile category), family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, and energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products (quartile category). Stratified analyses were undertaken according to dichotomous categories of cigarette smoking, alcohol consumption, body mass index, physical activity, and energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products. An interaction term was created by multiplying variables for pks status by those for dichotomous categories of each stratified variable, and its significance was statistically evaluated by the likelihood ratio test with 1 df.

In order to clarify factors associated with the prevalence of pks+E coli, risk factors of colorectal cancer and dietary intakes of food groups and nutrients were compared between participants with and without pks+E coli among the control group. Comparisons in mean, median, and proportions were tested with the t test, Wilcoxon rank-sum test, and χ² test, respectively. Furthermore, an unconditional logistic regression model was used to estimate ORs and 95% CIs of pks+E coli participants according to risk factors of colorectal cancer and quartile categories of energy-adjusted dietary intake. Linear trends for ORs in the logistic regression model were tested using the quartile categories as ordinal variables.

All reported p values are two-sided, and significance level was set at P < .05. All statistical analyses were undertaken using SAS 9.4 (SAS Institute Inc.).

3 | RESULTS

Table 1 presents participant characteristics by case-control status. The proportion of men was higher in cases than controls and cases were older, smoked more, and consumed more alcoholic beverages than controls. However, cases consumed fewer dairy products than controls. The distribution of other variables including body mass index, physical activity, and dietary intake except dairy products was similar between cases and controls.

We examined factors associated with the prevalence of pks+E coli among the control group (Tables S1–S6). No statistically
significant association was found for selected risk factors of colorectal cancer (Tables S1 and S2). Comparison of median intakes of food groups and nutrients showed no statistically significant difference between participants with and without \( \text{E coli}^+ \) among the control group except with regard to cruciferous vegetable intake (Tables S3 and S4); control participants with \( \text{E coli}^+ \) had significantly higher intake of cruciferous vegetables than those without, and energy-adjusted cruciferous vegetable intake was significantly associated with a higher prevalence of \( \text{E coli}^+ \) after adjustment for age, sex, and other lifestyle factors (Tables S3 and S5). In addition, energy-adjusted vitamin C intake was significantly associated with a higher prevalence of \( \text{E coli}^+ \), whereas energy-adjusted chromium intake was significantly associated with a lower prevalence (Table S6).

The proportion of \( \text{E coli}^+ \) was 32.6% among cases and 30.8% among controls. Table 2 shows ORs of the prevalence of colorectal neoplasia according to \( \text{E coli} \) status. Compared with the participants with \( \text{E coli}^- \), the OR (95% CI) for participants with \( \text{E coli}^+ \) was 1.04 (0.77-1.41) after adjusting for potential confounding factors. Although inclusion of dietary factors as potential confounding factors did not change the result, the same result was also obtained by further sensitivity analysis in which meat intake was replaced with red meat intake (data not shown). No significant association was observed regardless of sex and age group. Furthermore, site-specific analysis in 238 proximal, 179 distal, and 69 rectal cases following exclusion of 57 unclassified cases due to multiple lesions found no significant association regardless of site.

We reclassified colorectal neoplasia cases into colorectal cancer (\( n = 22 \)) and adenoma cases (\( n = 521 \)) and further defined advanced colorectal neoplasia (\( n = 102 \)) (Table 3). The proportion of \( \text{E coli}^+ \) was 40.9% for colorectal cancer, 32.2% for adenoma, and 26.5% for advanced colorectal neoplasia cases. Although no significant association was found for each outcome, the OR (95% CI) of colorectal cancer was 1.44 (0.48-4.26) among participants with \( \text{E coli}^+ \) compared with the participants with \( \text{E coli}^- \). Further analyses by rice, pickled vegetables, green and yellow vegetables, cruciferous vegetables, red meats, and total dietary fiber intake revealed a statistically significant interaction for cruciferous vegetable intake (\( P = .01 \) (Table S7).

**TABLE 1 Characteristics of study participants with colorectal neoplasia (cases) or normal colon (controls)**

|                          | Case                  | Control               | \( P \) value |
|--------------------------|-----------------------|-----------------------|---------------|
| Number                   | 543                   | 425                   |               |
| Men, n (%)               | 274 (50.5)            | 147 (34.6)            | <.010         |
| Age, years; mean (SD)    | 62.8 (9.8)            | 58.1 (11.3)           | <.010         |
| Body mass index, kg/m\(^2\); mean (SD) | 23.2 (3.3)            | 23.2 (3.5)            | .850          |
| Physical activity, metabolic equivalent-hours/day; mean (SD) | 40.8 (6.9) | 40.5 (5.8) | .530 |
| Current smokers, n (%)   | 115 (21.6)            | 58 (13.8)             | <.010         |
| Alcohol intake, ≥1 d/wk; n (%) | 277 (51.1)            | 170 (40.2)            | <.010         |
| Family history of colorectal cancer, yes; n (%) | 60 (11.1) | 39 (9.2) | .340 |
| Nonsteroidal anti-inflammatory drug use, yes; n (%) | 51 (9.4) | 48 (11.3) | .330 |

Dietary intake, median (interquartile range)

|                          | Case                  | Control               | \( P \) value |
|--------------------------|-----------------------|-----------------------|---------------|
| Energy, kcal/d           | 1418.8 (1076.2-1792.7)| 1328.0 (1050.5-1752.0)| .230          |
| Cereals, g/d             | 389.3 (296.4-507.1)   | 376.9 (296.7-483.6)   | .400          |
| Vegetables, g/d          | 81.9 (47.3-136.0)     | 90.0 (49.3-139.3)     | .240          |
| Fruits, g/d              | 48.7 (13.2-105.5)     | 52.0 (13.7-119.8)     | .280          |
| Meats, g/d               | 36.4 (17.9-64.8)      | 35.3 (19.4-67.0)      | .770          |
| Dairy products, g/d      | 42.9 (0-157.1)        | 50.0 (0-200.0)        | .045          |
TABLE 2 | Association of *Escherichia coli* containing polyketide synthase (pks-E coli) with the prevalence of colorectal neoplasia (colorectal cancer or adenoma)

| | pks-E coli | pks-E coli |
|---|---|---|
| All subjects | | |
| No. of cases | 366 | 177 |
| No. of controls | 294 | 131 |
| OR (95% CI)<sup>a</sup> | 1 (ref.) | 1.04 (0.78-1.39) |
| OR (95% CI)<sup>b</sup> | 1 (ref.) | 1.04 (0.77-1.41) |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.04 (0.77-1.41) |
| Sex | | |
| Men | | |
| No. of cases | 183 | 91 |
| No. of controls | 104 | 43 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.01 (0.61-1.67) |
| Women | | |
| No. of cases | 183 | 86 |
| No. of controls | 190 | 88 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 0.63 (0.29-1.40) |
| Age group | | |
| 40-49 y | | |
| No. of cases | 47 | 20 |
| No. of controls | 85 | 38 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.33 (0.64-2.73) |
| 50-59 y | | |
| No. of cases | 77 | 33 |
| No. of controls | 69 | 32 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.42 (0.80-2.50) |
| 60-69 y | | |
| No. of cases | 142 | 72 |
| No. of controls | 90 | 32 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.67 (0.33-1.37) |
| 70-79 y | | |
| No. of cases | 100 | 52 |
| No. of controls | 50 | 29 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.14 (0.78-1.67) |
| Tumor location | | |
| Proximal colon | | |
| No. of cases | 156 | 82 |
| No. of controls | 294 | 131 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.14 (0.78-1.67) |
| Distal colon | | |
| No. of cases | 125 | 54 |
| No. of controls | 294 | 131 |
| OR (95% CI)<sup>c</sup> | 1 (ref.) | 1.06 (0.69-1.62) |
| Rectum | | |
| No. of cases | 42 | 27 |

(Continues)

Abbreviations: CI, confidence interval; OR, odds ratio; ref., reference.

<sup>a</sup>Adjusted for sex and age (continuous).

<sup>b</sup>Further adjusted for cigarette smoking (never smokers, past smokers, <20, 20-39, ≥40 pack-years for current smokers), alcohol consumption (nondrinkers, past drinkers, occasional drinkers, <150, 150-299, 300-449, ≥450 g ethanol/wk for regular drinkers), body mass index (kg/m²) (<21, 21-23.9, 24-26.9, 27-29.9, ≥30), physical activity (metabolic equivalent-h/d, quartile category), family history of colorectal cancer (yes, no), and nonsteroidal anti-inflammatory drug use (yes, no).

<sup>c</sup>Further adjusted for energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products (quartile category).

### 4 | DISCUSSION

Overall, we found no statistically significant association between pks-E coli and the prevalence of colorectal adenoma lesions in the present cross-sectional analysis. This finding suggests that pks-E coli might not play a role in the early stage of the adenoma-carcinoma sequence. Stratified analyses by selected risk factors for colorectal cancer revealed effect modification by cereal and vegetable intake: a positive association was suggested only among participants who consumed cereals over the median intake or vegetables under the median intake.

Our findings, based on 521 colorectal adenoma cases, are consistent with a Japanese study that showed no statistically significant difference in the prevalence of pks-E coli between 37 colorectal adenoma cases (51%) and 26 controls (46%).<sup>14</sup> They are also consistent with a Swedish study that found a higher prevalence of pks-E coli among 134 colorectal adenoma cases (31.3%) than 65 controls (18.5%), although the difference was not statistically significant.<sup>13</sup> A statistically significant high prevalence of pks-E coli was observed among 25 patients with familial adenomatous polyposis (68%) compared to 23 controls (22%),<sup>24</sup> although this might rather suggest a different role of pks-E coli in the development of sporadic versus hereditary colorectal cancer.

Regarding the prevalence of pks-E coli among colorectal cancer cases, three studies from the UK, France, and Sweden found a statistically significant high prevalence compared to controls.<sup>11-13</sup> These studies involved 21, 38, and 39 cases, respectively, and had a prevalence between cases and controls of 67% and 21%, 55% and 19%, and 56.4% and 18.5%, respectively.<sup>11-13</sup> In contrast, no statistically significant difference in the prevalence of pks-E coli was observed between 35 colorectal cancer cases (43%) and 26 controls (46%) in Japan.<sup>14</sup> In addition, a study from Malaysia showed higher prevalence among 48 colorectal cancer cases (16.7%) than 23 controls (4.3%) but without statistical significance.<sup>15</sup> The two studies from Asian countries are in general agreement with our findings; however, our study included only 22 patients with colorectal cancer. Although the reason for these discrepant findings is unclear, one possible
TABLE 3 Association of Escherichia coli containing polyketide synthase (pks\(^+\) E coli) with the prevalence of colorectal cancer, adenoma, and advanced colorectal neoplasia\(^a\)

| Condition                        | pks\(^+\) E coli | pks\(^+\) E coli |
|----------------------------------|------------------|------------------|
| Colorectal cancer                |                  |                  |
| No. of cases                     | 13               | 9                |
| No. of controls                  | 294              | 131              |
| OR (95% CI)\(^b\)                | 1 (ref.)         | 1.44 (0.48-4.26) |
| Adenoma                          |                  |                  |
| No. of cases                     | 353              | 168              |
| No. of controls                  | 294              | 131              |
| OR (95% CI)\(^b\)                | 1 (ref.)         | 1.04 (0.76-1.41) |
| Advanced colorectal neoplasia\(^a\) |                  |                  |
| No. of cases                     | 75               | 27               |
| No. of controls                  | 294              | 131              |
| OR (95% CI)\(^b\)                | 1 (ref.)         | 0.85 (0.48-1.50) |

Abbreviations: CI, confidence interval; OR, odds ratio; ref., reference.

\(^a\)Advanced colorectal neoplasia comprised colorectal cancer and advanced adenoma (adenoma with a diameter ≥10 mm, high-grade dysplasia, or prominent villous component).

\(^b\)Adjusted for sex, age (continuous), cigarette smoking (never smokers, past smokers, <20, 20-39, ≥40 pack-years for current smokers), alcohol consumption (nondrinkers, past drinkers, occasional drinkers, <150, 150-299, 300-449, ≥450 g ethanol/wk for regular drinkers), body mass index (kg/m\(^2\)) (<21, 21-23.9, 24-26.9, 27-29.9, ≥30), physical activity (metabolic equivalent-h/d, quartile category), family history of colorectal cancer (yes, no), nonsteroidal anti-inflammatory drug use (yes, no), and energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products (quartile category).

The explanation is that the different prevalence of pks\(^+\) E coli among controls might reflect a difference in microbiota composition across populations and thus a difference in the production of colibactin. Of interest, prevalence in a previous Japanese study (46%) and our present study (30.8%) was higher than in studies from European countries (approximately 20%). If pks\(^+\) E coli among controls in the Japanese studies did not produce sufficient levels of colibactin for any reason, the presence of pks\(^+\) E coli would not necessarily imply colibactin exposure. Accordingly, further studies should examine the association between exposure to colibactin by pks\(^+\) E coli and the prevalence of colorectal neoplasia.

As pks\(^+\) E coli was detected in the gut of newborns, mother to offspring transmission during birth is suspected.\(^{25,28}\) Nevertheless, factors associated with long-term persistence have not been clarified. Further investigation of factors associated with the prevalence of pks\(^+\) E coli is needed, and might help our understanding of the difference in prevalence of pks\(^+\) E coli among populations. In this study, we found no statistically significant association of age, sex, and selected risk factors of colorectal cancer with the prevalence of pks\(^+\) E coli, suggesting that these are not major factors in the different prevalence of pks\(^+\) E coli among populations, as discussed above. However, we found that energy-adjusted intakes of cruciferous vegetables and vitamin C were significantly associated with higher prevalence, whereas energy-adjusted intake of chromium was significantly associated with lower prevalence. To our knowledge, our study is the second to examine the association between dietary factors and prevalence of pks\(^+\) E coli. The first study, undertaken in middle-aged Japanese, showed an inverse association between intake of green tea and manganese and the prevalence of pks\(^+\) E coli.\(^{27}\) These dietary factors are not known as established risk factors for colorectal cancer\(^{2,28}\) but could influence the pks status of E coli; however, the reported findings are inconsistent. Further accumulation of evidence is needed to clarify factors associated with the prevalence of pks\(^+\) E coli.

Given that some dietary risk factors for colorectal cancer are metabolized by the gut microbiota and might influence its composition,\(^{28}\) we hypothesized that they could modify the association between the pks status of E coli and the prevalence of colorectal neoplasia. Indeed, our stratified analyses by cereal, vegetable, and cruciferous vegetable intake found interactions. A stratified analysis by dietary fiber found no statistically significant interaction, although cereals and vegetables are rich in dietary fiber. A positive association was observed among participants who consumed cereals over the median intake and an inverse association was seen among those with

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**TABLE 4 Association of Escherichia coli containing polyketide synthase (pks\(^+\) E coli) with the prevalence of colorectal adenoma according to number of lesions**

| Lesion Count          | pks\(^+\) E coli | pks\(^+\) E coli |
|-----------------------|------------------|------------------|
| One adenoma           |                  |                  |
| No. of cases          | 162              | 90               |
| No. of controls       | 294              | 131              |
| OR (95% CI)\(^a\)     | 1 (ref.)         | 1.21 (0.85-1.73) |
| Two adenomas          |                  |                  |
| No. of cases          | 86               | 39               |
| No. of controls       | 294              | 131              |
| OR (95% CI)\(^a\)     | 1 (ref.)         | 1.15 (0.69-1.92) |
| Three or four adenomas|                  |                  |
| No. of cases          | 69               | 25               |
| No. of controls       | 294              | 131              |
| OR (95% CI)\(^a\)     | 1 (ref.)         | 0.71 (0.38-1.31) |
| More than five adenomas|                |                  |
| No. of cases          | 36               | 14               |
| No. of controls       | 294              | 131              |
| OR (95% CI)\(^a\)     | 1 (ref.)         | 0.97 (0.42-2.24) |

Abbreviations: CI, confidence interval; OR, odds ratio; ref., reference.

\(^a\)Adjusted for sex, age (continuous), cigarette smoking (never smokers, past smokers, <20, 20-39, ≥40 pack-years for current smokers), alcohol consumption (nondrinkers, past drinkers, occasional drinkers, <150, 150-299, 300-449, ≥450 g ethanol/wk for regular drinkers), body mass index (kg/m\(^2\)) (<21, 21-23.9, 24-26.9, 27-29.9, ≥30), physical activity (metabolic equivalent-h/d, quartile category), family history of colorectal cancer (yes, no), nonsteroidal anti-inflammatory drug use (yes, no), and energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products (quartile category).
| Smoking status        | \( pks^- E\ coli \) | \( pks^+ E\ coli \) | \( P \) for interaction |
|-----------------------|---------------------|---------------------|--------------------------|
| **Never smokers**     |                     |                     |                          |
| No. of cases          | 142                 | 80                  |                          |
| No. of controls       | 153                 | 76                  |                          |
| OR (95% CI)\(^a\)     | 1 (ref.)            | 0.96 (0.63-1.47)    |                          |
| **Ever smokers**      |                     |                     |                          |
| No. of cases          | 216                 | 94                  |                          |
| No. of controls       | 137                 | 54                  |                          |
| OR (95% CI)\(^a\)     | 1 (ref.)            | 1.17 (0.74-1.84)    |                          |

| Alcohol intake       | .380                |                     |                          |
|----------------------|---------------------|---------------------|--------------------------|
| **Nondrinkers**      |                     |                     |                          |
| No. of cases         | 153                 | 80                  |                          |
| No. of controls      | 158                 | 70                  |                          |
| OR (95% CI)\(^a\)    | 1 (ref.)            | 1.24 (0.80-1.90)    |                          |
| **Drinkers**         |                     |                     |                          |
| No. of cases         | 213                 | 96                  |                          |
| No. of controls      | 135                 | 60                  |                          |
| OR (95% CI)\(^a\)    | 1 (ref.)            | 0.87 (0.55-1.36)    |                          |

| Body mass index (kg/m\(^2\)) | .610 |                     |                          |
|-------------------------------|-----|---------------------|--------------------------|
| \(<25\)                       |     |                     |                          |
| No. of cases                  | 267 | 127                 |                          |
| No. of controls                | 215 | 98                  |                          |
| OR (95% CI)\(^a\)             | 1 (ref.) | 1.14 (0.80-1.63)    |                          |
| \(\geq25\)                    |     |                     |                          |
| No. of cases                  | 93  | 44                  |                          |
| No. of controls                | 74  | 31                  |                          |
| OR (95% CI)\(^a\)             | 1 (ref.) | 0.96 (0.49-1.89)    |                          |

| Physical activity (metabolic equivalent-h/d) | .310 |                     |                          |
|---------------------------------------------|-----|---------------------|--------------------------|
| \(<Median\)                                |     |                     |                          |
| No. of cases                                | 176 | 104                 |                          |
| No. of controls                             | 142 | 69                  |                          |
| OR (95% CI)\(^a\)                          | 1 (ref.) | 1.22 (0.79-1.88)    |                          |
| \(\geq\) Median                            |     |                     |                          |
| No. of cases                                | 190 | 73                  |                          |
| No. of controls                             | 150 | 62                  |                          |
| OR (95% CI)\(^a\)                          | 1 (ref.) | 0.88 (0.55-1.39)    |                          |

| Energy-adjusted cereal intake\(^b\)        | .002 |                     |                          |
|---------------------------------------------|-----|---------------------|--------------------------|
| \(<Median\)                                |     |                     |                          |
| No. of cases                                | 201 | 75                  |                          |
| No. of controls                             | 140 | 72                  |                          |
| OR (95% CI)\(^a\)                          | 1 (ref.) | 0.66 (0.43-1.03)    |                          |
| \(\geq\) Median                            |     |                     |                          |
| No. of cases                                | 165 | 102                 |                          |
| No. of controls                             | 154 | 59                  |                          |

(Continues)
### Table 5 (Continued)

|                        | pks<sup>−</sup> E coli | pks<sup>+</sup> E coli | P for interaction |
|------------------------|------------------------|------------------------|-------------------|
| OR (95% CI)<sup>a</sup> | 1 (ref.)               | 1.66 (1.05-2.61)       | .080              |

**Energy-adjusted vegetable intake**<sup>b</sup>

|                     | No. of cases | No. of controls | OR (95% CI)<sup>a</sup> |
|---------------------|--------------|-----------------|--------------------------|
| <Median             |              |                 |                          |
| No. of cases        | 195          | 155             | 1.33 (0.84-2.10)         |
| No. of controls     | 97           | 57              |                          |
| ≥Median             |              |                 |                          |
| No. of cases        | 171          | 139             | 0.81 (0.52-1.26)         |
| No. of controls     | 80           | 74              |                          |

**Energy-adjusted fruit intake**<sup>b</sup>

|                     | No. of cases | No. of controls | OR (95% CI)<sup>a</sup> |
|---------------------|--------------|-----------------|--------------------------|
| <Median             |              |                 |                          |
| No. of cases        | 203          | 149             | 0.83 (0.53-1.31)         |
| No. of controls     | 82           | 63              |                          |
| ≥Median             |              |                 |                          |
| No. of cases        | 163          | 145             | 1.32 (0.84-2.06)         |
| No. of controls     | 95           | 68              |                          |

**Energy-adjusted meat intake**<sup>b</sup>

|                     | No. of cases | No. of controls | OR (95% CI)<sup>a</sup> |
|---------------------|--------------|-----------------|--------------------------|
| <Median             |              |                 |                          |
| No. of cases        | 189          | 147             | 1.23 (0.80-1.89)         |
| No. of controls     | 98           | 65              |                          |
| ≥Median             |              |                 |                          |
| No. of cases        | 177          | 147             | 0.94 (0.59-1.50)         |
| No. of controls     | 79           | 66              |                          |

**Energy-adjusted dairy product intake**<sup>b</sup>

|                     | No. of cases | No. of controls | OR (95% CI)<sup>a</sup> |
|---------------------|--------------|-----------------|--------------------------|
| <Median             |              |                 |                          |
| No. of cases        | 191          | 141             | 1.10 (0.72-1.70)         |
| No. of controls     | 104          | 71              |                          |
| ≥Median             |              |                 |                          |
| No. of cases        | 175          | 153             | 1.00 (0.63-1.58)         |
| No. of controls     | 73           | 60              |                          |

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**Abbreviations:** CI, confidence interval; OR, odds ratio; ref., reference.

<sup>a</sup>Adjusted for sex, age (continuous), cigarette smoking (never smokers, past smokers, <20, 20-39, ≥40 pack-years for current smokers), alcohol consumption (nondrinkers, past drinkers, occasional drinkers, <150, 150-299, 300-449, ≥450 g ethanol/wk for regular drinkers), body mass index (kg/m<sup>2</sup>) (<21, 21-23.9, 24-26.9, 27-29.9, ≥30), physical activity (metabolic equivalent-h/d, quartile category), family history of colorectal cancer (yes, no), nonsteroidal anti-inflammatory drug use (yes, no), and energy-adjusted intakes of cereals, vegetables, fruits, meats, and dairy products (quartile category).

<sup>b</sup>Cereal intake includes seven items: cooked rice, grain, millet, sawa millet, bread [including pastry], udon, soba, and brown rice. Vegetable intake includes 15 items: carrot, spinach, pumpkin, salted pickles of Chinese radish, green leafy vegetables, Chinese cabbage, cucumber, and eggplant, tomato, Welsh onion, garland chrysanthemum, broccoli, onion, Chinese cabbage, and tomato juice. Fruit intake includes six items: mandarin orange, apple, watermelon, banana, orange juice, and salted pickles of plum. Meat intake includes 12 items: steaks, grilled beef, stir-fried pork, stewed beef, stir-fried pork, deep-fried pork, stewed pork, western-style stewed pork, Japanese-style pork in soup, pork liver, deep-fried chicken, and chicken liver. Dairy product intake includes two items: whole milk and low-fat milk.
consumption under the median intake. The opposite direction of associations was observed for vegetable and cruciferous vegetable intake. The reasons for the positive association among participants who consumed cereals over the median intake or vegetables under the median intake, particularly cruciferous vegetables, are unclear. As dietary fiber did not modify the association in this study, factors other than dietary fiber might be considered. Cereals are also high glycemic index foods and major sources of dietary carbohydrates, including starch, which have been associated with chronic conditions such as obesity and diabetes.29,30 Thus, we speculate that a high cereal or low vegetable intake might imply a somewhat unhealthy diet, and an intestinal environment characterized by relatively high cereal or low vegetable intake might promote carcinogenesis due to pks⁺ E. coli. As this is the first report of such interaction, confirmation in other studies is warranted.

The strengths of our study include its relatively large number of adenoma cases (n = 521) and adjustment for potential confounders, including dietary factors. In addition, all participants underwent total colonoscopy, reducing the possibility of misclassification of case and control status. Nevertheless, several limitations need to be addressed. First is the cross-sectional design, and the possibility that the observed associations were subject to reverse causality. Second, our study cohort was mainly derived from a single small island in Japan, which might limit our representativeness. Considering that large variation in microbiota composition among individuals is likely, primarily due to different external environmental factors, the generalizability of our finding to other populations is also limited. Finally, although we adjusted for known or potential confounding factors in the multivariable models, residual or unmeasured confounding remains possible.

In conclusion, we found that the pks status of E. coli was not significantly associated with the prevalence of colorectal adenoma lesions in a cross-sectional analysis in a Japanese cohort. However, positive associations were suggested under certain intake level of cereals and vegetables, and thus dietary intake might modify this association. Further studies using an appropriate biomarker of pks⁺ E. coli exposure from a large number of colorectal neoplasia cases are required.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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