Risk factors for esophageal iodine-unstained lesions and changing trends among Japanese alcohol-dependent men (2003-2018)

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Abstract
Globally, a decreasing incidence of male esophageal squamous cell carcinoma (ESCC) has been observed in recent decades. We evaluated the determinants of esophageal distinct iodine-unstained lesions (DIULs), high-cancer-risk lesions and ESCC, among 3858 Japanese alcohol-dependent men (40-79 years) who underwent chromoendoscopic screening between 2003 and 2018. The initial screening detected DIULs ≥ 5 mm in 541 patients (dysplasia in 319 and SCC in 129) and multiple DIULs in 640. The detection rates for DIULs and chronic atrophic gastritis (CAG), pack-years, and the mean corpuscular volume (MCV) decreased over the course of the study period, while the detection of hiatal hernia and/or columnar-lined esophagus (HH/CLE) and the carriers of inactive heterozygous aldehyde dehydrogenase-2 (ALDH2, rs671) increased. Multiple logistic regression analyses showed that an older age, larger number of pack-years, smaller body mass index, larger MCV, presence of a slow-metabolizing alcohol dehydrogenase-1B genotype (rs1229984), presence of an inactive heterozygous ALDH2 genotype, and more advanced degree of CAG increased the odds ratios (ORs) for DIULs, while the 2008-2013 and 2014-2018 screening periods had lower ORs for DIULs than the 2003-2007 screening period. The presence of HH/CLE decreased the OR for multiple DIULs and was associated with a more proximal location of ESCC. In conclusion, the detection of DIULs in an alcohol-dependent population decreased between 2003 and 2018. In addition to reported determinants of ESCC, CAG and HH/CLE were associated with the risk of DIULs. Enigmatically, however, the decline in the detection of DIULs was not adequately explained by these factors and warrants further research.

Keywords
alcohol dependence, chronic atrophic gastritis, esophageal cancer, esophageal dysplasia, hiatal hernia
1 | INTRODUCTION

Squamous cell dysplasia and carcinoma in the upper aerodigestive tract as well as chronic atrophic gastritis (CAG) and gastric adenocarcinoma are frequently detected by chromoendoscopic screening using esophageal iodine staining in Japanese alcohol-dependent men.1-4 Esophageal dysplasia and esophageal squamous cell carcinoma (ESCC) were histologically diagnosed in 88% of mucosal biopsy specimens of distinct iodine-unstained lesions (DIULs) with a greatest dimension of 5 mm or more.5 The presence of multiple esophageal DIULs is also frequently found in this population.5 The presence of DIULs ≥ 5 mm5 and multiple DIULs6-9 indicate a high risk of multicentric cancerization in the upper aerodigestive tract among Japanese drinkers.

Inactive aldehyde dehydrogenase-2 encoded by the ALDH2*1/*2 genotype (rs671) and slow-metabolizing alcohol dehydrogenase-1B encoded by the ADH1B*1/*1 genotype (rs1229984) are strong risk factors for ESCC.1-6,10-12 DIULs ≥ 5 mm,3,13 and multiple DIULs3,6,13,14 among East Asian drinkers.

We recently reported that the detection rate of ESCC as well as that of CAG and gastric adenocarcinoma decreased markedly between 2003 and 2018 in an alcohol-dependent population.6 The reduction in Helicobacter pylori infection is the main reason for the reductions in CAG and gastric cancer.15 Growing evidence has shown that the presence of CAG increases the risk of ESCC.16-24 A meta-analysis of seven studies has shown a 1.94-fold higher relative risk of ESCC among people with CAG.25 We have also demonstrated a high risk of ESCC among Japanese alcohol-dependent men with CAG.4,25 The reduction in ESCC in this population might also be linked to global trends toward a decreasing incidence of male ESCC during recent decades.26

The aims of this study were to evaluate the trends in the detection of large DIULs and multiple DIULs, which are regarded as high-cancer-risk lesions, between 2003 and 2018 and to clarify the factors associated with DIULs among Japanese alcohol-dependent men.

2 | MATERIALS AND METHODS

2.1 | Subjects

The subjects were 3858 Japanese alcohol-dependent men aged 40-79 years old who (a) visited the Kurihama Medical and Addiction Center for the treatment of alcohol dependence, (b) did not have any history of upper aerodigestive tract or gastric cancer treatment or a gastrectomy, and (c) underwent endoscopic screening with esophageal iodine staining between 2003 and 2018. We used only the initial screening results, and there was no overlap among the subjects.

All the subjects met the DSM-IIIR, DSM-IV, or ICD-10 criteria for alcohol dependence.27-29 Each subject was asked about his usual alcohol consumption during the preceding year and smoking habits using a structured questionnaire, as previously reported.1,3 The proposal for this study was approved by the ethics committee of the Kurihama Medical and Addiction Center. The ethics committee determined that the requirement for additional informed consent to participate in the present study was waived because of its retrospective design, and patients were able to exclude themselves using the opt-out method on the Center’s website.

2.2 | Endoscopic screening

The examinations were performed using Olympus endoscopes (models XQ230, Q240, Q240Z, Q260, and Q260Z in chronological order of use; Olympus Optical Co. Ltd.). Almost all the screening examinations were performed by a single endoscopist (A. Yokoyama) or were performed under his supervision. The screening program and diagnostic procedure have been described in previous reports.1,3 A 3% iodine solution was consistently used for esophageal iodine staining during the study period. Mucosal biopsy specimens were collected from DIULs whose greatest diameter was 5 mm or more (Figure 1A). Multiple DIULs were recorded when 10 or more DIULs of any size were observed in at least one endoscopic field of view (Figure 1B). The center of each ESCC lesion was defined as “the distance from the incisor to the proximal end of the DIUL” plus “half of the axial diameter of the DIUL.” Using digitalized images stored within a medical imaging communication system, a single endoscopist (A. Yokoyama) reviewed the endoscopic findings for the hiatal hernia and/or columnar-lined esophagus (HH/CLE) and CAG. When the axial length between the diaphragmatic pinch and the border between the white squamous epithelium and the red epithelium was ≥10 mm for the entire circumference (Figure 1C), the subject was diagnosed as having an HH/CLE ≥ 10 mm, which was regarded as evidence of long-term gastroesophageal reflux. A digitalized record of the area around the esophagogastric junction was absent in 37 subjects. According to the Kimura-Takemoto classification system for CAG (Figure 1D),30 no atrophy was classified as C0. Atrophic mucosa limited to the antrum, the gastric angle or the lower corpus, the upper corpus, or the gastric cardia surroundings with maintained folds in the greater curvature was classified as C1, C2, C3, or O1, respectively. Atrophy in the entire stomach with a lack of folds in the greater curvature was classified as O3, while O2 represented an intermediate designation between O1 and O3. The patients were classified into three categories (C0 to C2, C3 to O1, and O2-O3) because the gastric cancer detection rate reportedly increase in a stepwise manner according to these categories.31

2.3 | Evaluation of H. pylori infection

In February 2013, the Japanese universal health insurance plan was expanded to include coverage for H. pylori eradication therapy for
Since then, the presence of *H. pylori* infection has been examined using the stool antigen test (Testmate Rapid Pylori Antigen; Wakamoto Pharmaceutical) and the (13)C-urea breath test (BML, Ltd.) in patients who did not have a history of *H. pylori* eradication and wished to undergo testing.

### 2.4 Mean corpuscular volume

During each patient's initial visit to the Center for the treatment of alcohol dependence, we measured the mean corpuscular volume (MCV) using the electrical impedance method with an autoanalyzer (CELL-DYN 3500; Abbott). We dichotomized the results into an MCV < 106 fl group and an MCV ≥ 106 fl group because macrocytosis with an MCV value ≥ 106 fl was found to be associated with an increased risk of ESCC in our previous studies of alcohol-dependent men and men who had undergone an endoscopic mucosectomy for early ESCC.

### 2.5 ALDH2 and ADH1B genotyping

We had previously determined the ALDH2 and ADH1B genotypes of 3335 subjects from whom written informed consent had been obtained for the study of ALDH2 and ADH1B genotype-associated phenotypes and comorbidities, which had been approved by the ethics committee of the Center. PCR-restriction fragment length polymorphism methods were used to genotype ALDH2 and ADH1B in DNA obtained from blood samples.

### 2.6 Statistical analysis

Values were expressed as the mean and standard deviation (SD) or the percentage. Age was compared using the χ² test or the t test between two groups, and trends were tested using a linear regression model. Age-adjusted P-values for other variables were calculated using an analysis of the covariance, a multiple linear regression model, or the Cochran-Mantel-Haenszel test, as appropriate. The multivariate odds ratios (ORs) and the 95% confidence intervals (CIs) were calculated using multiple logistic models. We combined the ADH1B*1/*2 genotype carriers and the ADH1B*2/*2 genotype carriers into a single group because of the semidominant nature of the ADH1B*2 allele. Statistical significance was defined as a P-value of <0.05. All the statistical analyses were performed using SAS software (version 9.4; SAS Institute).

### 3 RESULTS

DIULs ≥ 5 mm and multiple DIULs were diagnosed in 541 (14.0%) and 640 (16.6%) subjects, respectively. A targeted biopsy of DIULs ≥ 5 mm was performed in 471 subjects, and esophageal squamous cell dysplasia and ESCC were diagnosed in 319 and 129 subjects, respectively. The main reasons for not performing a biopsy were the presence of a bleeding tendency and/or liver cirrhosis.

Table 1 shows the background factors according to whether the DIULs were ≥5 mm (all), whether the DIULs were ≥5 mm (dysplasia), whether the DIULs were ≥5 mm (SCC), and whether multiple...
TABLE 1  Background characteristics of Japanese alcohol-dependent men according to the results of esophageal iodine staining

|                      | DIULs ≥ 5 mm |                      |                      |                      |
|----------------------|--------------|----------------------|----------------------|----------------------|
|                      | Absent       | Present              | Dysplasia            | SCC                   |
|                      | N = 3317     | N = 541              | N = 319              | N = 129              |
| Age (years old) (n, %) |              |                      |                      |                      |
| 40-49                | 1107 (33.4)  | 69 (12.8)            | 42 (13.2)            | 15 (11.6)            |
| 50-59                | 1153 (34.8)  | 207 (38.3)           | 121 (37.9)           | 47 (36.4)            |
| 60-69                | 755 (22.8)   | 198 (36.6)           | 120 (37.6)           | 48 (37.2)            |
| 70-79                | 302 (9.1)    | 67 (12.4)            | 36 (11.3)            | 19 (14.7)            |
| Mean ± SD            | 55.1 ± 9.6   | 59.4 ± 8.3           | 59.3 ± 8.3           | 60.1 ± 8.6           |
| Alcohol intake (g ethanol/d) |          |                      |                      |                      |
| Mean ± SD            | 119.1 ± 74.6 | 111.4 ± 62.7         | 109.1 ± 58.9         | 107.4 ± 49.9         |
| Pack-years           | N = 3310     | N = 541              | N = 319              | N = 129              |
| Mean ± SD            | 30.8 ± 23.1  | 36.3 ± 24.9          | 37.0 ± 25.3          | 35.7 ± 24.0          |
| Body mass index (kg/m²) | N = 3239     | N = 526              | N = 310              | N = 123              |
| Mean ± SD            | 22.0 ± 3.5   | 21.1 ± 3.2           | 20.8 ± 3.1           | 21.2 ± 3.1           |
| MCV                  | N = 3275     | N = 534              | N = 316              | N = 128              |
| Mean ± SD            | 99.9 ± 8.0   | 102.9 ± 8.5          | 102.9 ± 8.2          | 103.8 ± 8.7          |
| MCV ≥ 106 fl         | 706 (21.6)   | 178 (33.3)           | 102 (32.3)           | 49 (38.3)            |
| ALDH2 genotype (n, %) |              |                      |                      |                      |
| *1/*1 (active)       | 2496 (87.3)  | 319 (67.0)           | 201 (74.4)           | 54 (42.2)            |
| *1/*2 (inactive)     | 363 (12.7)   | 157 (33.0)           | 69 (25.6)            | 74 (57.8)            |
| ADH1B genotype (n, %) |              |                      |                      |                      |
| *1/*1 (slow)         | 760 (26.6)   | 191 (40.1)           | 101 (37.4)           | 60 (46.9)            |
| *1/*2 or *2/*2 (fast) | 2099 (73.4)  | 285 (59.9)           | 169 (62.6)           | 68 (53.1)            |
| HH/CLE ≥ 10 mm       |                      |                      |                      |                      |
| Absent               | 2308 (70.3)  | 413 (76.8)           | 246 (77.6)           | 93 (72.7)            |
| Present              | 975 (29.7)   | 125 (23.2)           | 71 (22.4)            | 35 (27.3)            |
| Chronic atrophic gastritis (n, %) |   |                      |                      |                      |
| C0-C2                | 2266 (68.3)  | 275 (50.8)           | 162 (50.8)           | 63 (48.8)            |
| C3-O1                | 754 (22.7)   | 172 (31.8)           | 105 (32.9)           | 37 (28.7)            |
| O2-O3                | 297 (9.0)    | 94 (17.4)            | 52 (16.3)            | 29 (22.5)            |

Note: P values were tested between the DIUL-absent and -present groups. Note: Age was compared by χ² test or t test between groups. For other variables, P-values were adjusted for age by analysis of covariance for mean values and by Cochran-Mantel-Haenszel test for categorical data (for trend for chronic atrophic gastritis).

Abbreviations: ADH1B, alcohol dehydrogenase-1B; ALDH2, aldehyde dehydrogenase-2; DIULs, distinct iodine-unstained lesions; HH/CLE, hiatal hernia and/or columnar-lined esophagus; MCV, mean corpuscular volume; SCC, squamous cell carcinoma.

*<0.05.
†<0.0005.
‡<0.0001.

DIULs were present. As a significantly older age was observed in the DIULs-present groups, compared with the DIULs-absent groups, all the subsequent statistical analyses were performed with age adjustments. An MCV ≥ 106 fl, the inactive heterozygous ADH1B*1/*2 genotype, the slow-metabolizing ADH1B*1/*1 genotype, and advanced degrees of CAG were more frequently found in all the DIULs-present groups. A larger number of pack-years, a smaller body mass index (BMI), and a lower frequency of HH/CLE ≥ 10 mm were observed in all the DIULs-present groups except for the ESCC group.

For the 2003-2007 period, the 2008-2012 period, and the 2013-2018 period, the respective detection rates of DIULs ≥ 5 mm (all:
20.6%, 12.4%, and 8.6%; dysplasia: 11.7%, 7.9%, and 4.8%; SCC: 4.4%, 3.1%, and 2.4%) and multiple DIULs (20.0%, 14.9%, and 14.9%) decreased (Table 2). The incidence of advanced CAG also decreased, while that of HH/CLE ≥ 10 mm increased, during the study period. No significant differences in age, daily alcohol consumption, BMI, or genotype distribution of ADH1B were seen during the study period. The number of pack-years, mean MCV, and the frequency of macrocytosis with an MCV ≥ 106 fl all decreased. The frequency of inactive ALDH2*1/*2 increased from 13.9% to 15.1% and 17.8% for the respective time periods.

The distance of the ESCC lesion from the incisors, the ESCC depth, and the intraesophageal multiplicity of ESCCs were compared according to whether an HH/CLE ≥ 10 mm was present or according to the degree of CAG (Table 3). ESCC lesions were located in more proximal sites of the esophagus in subjects with an HH/CLE ≥ 10 mm. An HH/CLE ≥ 10 mm was more frequently accompanied by a milder degree of CAG.

Table 4 shows the associations of the H. pylori status with CAG and HH/CLE during the 2013-2018 period. CAG classification was strongly associated with H. pylori infection. A high rate...
of *H. pylori*-negative results was observed with the C0-C1 classification, while a low rate was observed with the C2-O1 classification. The rate was intermediate for the O2-O3 classification. HH/CLE ≥ 10 mm was found more frequently among *H. pylori*-negative patients.

Multiple logistic regression analyses showed that the C3-O1 category and the O2-O3 category of CAG increased the ORs (95% CI) for DIULs ≥ 5 mm (all: 1.52 [1.18-1.90] and 1.76 [1.27-2.44], respectively; dysplasia: 1.57 [1.15-2.15] and 1.72 [1.15-2.58], respectively; SCC: 1.56 [0.96-2.54] and 2.27 [1.29-3.99]) and multiple DIULs (1.40 [1.10-1.79] and 1.67 [1.22-2.30], respectively), compared with the C0-C2 category (Table 5). The presence of HH/CLE ≥ 10 mm decreased the OR for multiple DIULs (0.71 [0.56-0.91]). Screening during the 2008-2012 period and the 20013-2018 period showed a decrease in the ORs in all the DIULs-present groups, compared with the 2003-2007 period. An older age and the presence of the *ALDH2*1/*2 and *ADH1B*1/*1 genotypes increased the ORs in all the DIULs-present groups, while a larger BMI decreased the ORs. An MCV ≥ 106 fl increased the ORs in the DIULs-present groups except for the ESCC group, while a smaller number of pack-years decreased the ORs for DIULs ≥ 5 mm (dysplasia) and multiple DIULs.

After excluding the 129 ESCC patients, 234 patients had DIULs ≥ 5 mm and multiple DIULs simultaneously. The abovementioned backgrounds of these 234 patients were compared with those of 178 patients with DIULs ≥ 5 mm alone and 303 patients with multiple DIULs alone. A multiple linear regression analysis using a stepwise procedure and \( P < 0.05 \) as the criterion for entry and removal showed that a 10-year increment of age, an *ALDH2*1/*2 genotype, and an *ADH1B*1/*1 genotype increased the ORs (95% CI) for the simultaneous presence of DIULs ≥ 5 mm and multiple DIULs (1.78 [1.31-2.40], 4.43 [2.38-8.25], and 2.29 [1.39-3.77], respectively), compared with the presence of DIULs ≥ 5 mm alone. A comparison of the simultaneous presence of DIULs ≥ 5 mm and multiple DIULs with the presence of multiple DIULs alone did not select any significant background factors.

### TABLE 3 Esophageal SCC, HH/CLE, and chronic atrophic gastritis in Japanese alcohol-dependent men

| HH/CLE ≥ 10 mm | Chronic atrophic gastritis |
|----------------|---------------------------|
|                | C0-C2 | C3-O1 | O2-O3 | P   |
| Most proximal SCC lesion |       |       |       |     |
| <30.4 cm from incisor | 25 (58.1) | 18 (41.9) |       | .010 |
| 30.4-34.5 cm from incisor | 33 (76.7) | 10 (23.3) |       | .614 |
| >34.5 cm from incisor | 35 (83.3) | 7 (16.7) |       | .041 |
| Mean ± SD (cm) | 32.7 ± 5.7 | 30.4 ± 5.8 |       | .17  |
| Most distal SCC lesion |       |       |       |     |
| <30.4 cm from incisor | 27 (62.8) | 16 (37.2) |       | .061 |
| 30.4-36.0 cm from incisor | 32 (74.4) | 11 (25.6) |       | .250 |
| >36.0 cm from incisor | 34 (81.0) | 8 (19.0) |       | .20  |
| Mean ± SD (cm) | 34.0 ± 5.5 | 31.7 ± 5.9 |       | .28  |
| SCC depth |       |       |       |     |
| Intraepithelium | 43 (72.9) | 16 (27.1) |       | .56  |
| Proper mucosal layer | 16 (66.7) | 8 (33.3) |       | .250 |
| Muscularis mucosa | 11 (66.7) | 5 (33.3) |       | .56  |
| Submucosa | 11 (73.3) | 4 (26.7) |       | .02  |
| Beyond proper muscle layer | 12 (85.7) | 2 (14.3) |       | .02  |
| Multiple intraesophageal SCCs |       |       |       |     |
| Absence | 72 (74.2) | 25 (25.8) |       | .48  |
| Presence | 21 (67.7) | 10 (32.3) |       | .13  |
| Chronic atrophic gastritis (n, %) |       |       |       |     |
| C0-C2 | 1635 (65.0) | 882 (35.0) |       |     |
| C3-O1 | 744 (81.3) | 171 (18.7) |       |     |
| O2-O3 | 342 (87.9) | 47 (12.1) |       |     |

Note: P-values were for the trend for chronic atrophic gastritis and for the homogeneity for HH/CLE, where age was adjusted using a multiple linear regression model for continuous variables and by the Cochran-Mantel-Haenszel test for categorical variables.

Abbreviations: ADH1B, alcohol dehydrogenase-1B; ALDH2, aldehyde dehydrogenase-2; HH/CLE, hiatal hernia and/or columnar-lined esophagus; SCC, squamous cell carcinoma.
The detection rates of DIULs ≥ 5 mm (all), DIULs ≥ 5 mm (dysplasia), and multiple DIULs, which are regarded as high-ESCC-risk lesions, and of ESCC were relatively high during the chromoendoscopic screening of alcohol-dependent men. The rates of all DIULs and advanced CAG decreased, and the rate of HH/CLE ≥ 10 mm increased during the study period. The CAG classification was strongly associated with the status of *H. pylori* infection. The relatively high rate of negative results for *H. pylori* infection with O2-O3 atrophy probably reflects previous *H. pylori* infection including the spontaneous disappearance of *H. pylori* because of severe atrophy.37 The reduction in *H. pylori* infection in Japan is the main reason for the decrease in the detection of advanced CAG over time.15,32 Advanced CAG increased the ORs of all the DIULs, and the presence of HH/CLE ≥ 10 mm decreased the OR of the multiple DIULs. Other factors associated with the DIULs were an older age, a larger number of pack-years, a smaller BMI, a larger MCV, the presence of a slow-metabolizing ADH1B genotype, and the presence of an inactive heterozygous ALDH2 genotype.

There is growing evidence of a positive association between CAG and the risk of ESCC.4,16-25 Oral microflora form acetaldehyde, a carcinogen for ESCC, from ethanol and contribute to the high acetaldehyde levels in saliva after ethanol ingestion.38,39 An overgrowth of oral microflora and high acetaldehyde production in the saliva is observed in alcohol-dependent patients.39,40 Saliva containing microflora are transported from the mouth to the esophagus and stomach. The oral bacterial overgrowth in the esophagus and hypochlorhydric stomach with CAG may increase local acetaldehyde production.41

### TABLE 4

**Association of *H. pylori* infection status with chronic atrophic gastritis and HH/CLE in Japanese alcohol-dependent men (2013-2018)**

|                        | N   | *H. pylori* infection |          |          |          |          |          |          |
|------------------------|-----|-----------------------|----------|----------|----------|----------|----------|----------|
|                        |     | Negative              | Positive | P value  | Present  |          |          |          |
| Chronic atrophic gastritis |     |                       |          |          |          |          |          |          |
| C0-C1                  | 330 | 293 (88.8%)           | 37 (11.2%)| <.0001   |          |          |          |          |
| C2                     | 114 | 24 (21.1%)            | 90 (78.9%)|          |          |          |          |          |
| C3                     | 93  | 10 (10.8%)            | 83 (89.2%)|          |          |          |          |          |
| O1                     | 55  | 7 (12.7%)             | 48 (87.3%)|          |          |          |          |          |
| O2                     | 40  | 14 (35.0%)            | 26 (65.0%)|          |          |          |          |          |
| O3                     | 16  | 6 (37.5%)             | 10 (62.5%)|          |          |          |          |          |
| HH/CLE ≥ 10 mm          |     |                       |          |          |          |          |          |          |
| Absent                 | 437 | 226 (51.7%)           | 211 (48.3%)| .022     |          |          |          |          |
| Present                | 208 | 128 (61.5%)           | 80 (38.5%)|          |          |          |          |          |

Note: *H. pylori* infection status was examined using the stool antigen test and the (13)C-urea breath test in the patients without a history of *H. pylori* eradication. *P*-values were examined by χ² test.

Abbreviation: HH/CLE, hiatal hernia and/or columnar-lined esophagus.

### TABLE 5

**Multiple logistic analyses for identifying determinants of esophageal DIULs in Japanese alcohol-dependent men**

|                        | DIULs ≥ 5 mm (all) | DIULs ≥ 5 mm (dysplasia) | DIULs ≥ 5 mm (SCC) | Multiple DIULs |
|------------------------|--------------------|--------------------------|--------------------|----------------|
|                        | OR (95% CI)        | OR (95% CI)              | OR (95% CI)        | OR (95% CI)    |
| Age (per +10 y)        | 1.67 (1.47-1.90)   | 1.52 (1.29-1.78)         | 1.81 (1.43-2.29)   | 1.70 (1.50-1.92) |
| Alcohol intake (per +22 g ethanol/day) | 0.99 (0.96-1.03) | 0.97 (0.93-1.02) | 0.98 (0.91-1.05) | 0.97 (0.94-1.01) |
| Pack-years (per −10 pack-years) | 0.96 (0.92-1.004) | 0.95 (0.90-0.998) | 0.96 (0.89-1.05) | 0.95 (0.92-0.99) |
| Body mass index (per +1 kg/m²) | 0.92 (0.89-0.95) | 0.89 (0.85-0.93) | 0.93 (0.87-0.99) | 0.94 (0.91-0.97) |
| MCV ≥ 106 fl vs <106 fl | 1.50 (1.18-1.90) | 1.59 (1.18-2.14) | 1.20 (0.77-1.88) | 1.72 (1.37-2.17) |
| ALDH2*1/*2 carriers vs. *1/*1 carriers | 3.44 (2.67-4.43) | 2.28 (1.65-3.16) | 10.1 (6.65-15.5) | 4.30 (3.39-5.46) |
| ADH1B*1/*1 carriers vs *2 carriers | 2.68 (2.12-3.39) | 2.45 (1.83-3.28) | 3.50 (2.29-5.35) | 3.47 (2.78-4.34) |
| HH/CLE ≥ 10 mm         | 0.94 (0.73-1.21)   | 0.88 (0.64-1.21)         | 1.13 (0.72-1.78)   | 0.71 (0.56-0.91) |
| Chronic atrophic gastritis |                     |                          |                    |                |
| C0-C1                  | 1 (referent)       | 1 (referent)             | 1 (referent)       | 1 (referent)   |
| C3-O1                  | 1.52 (1.18-1.96)   | 1.57 (1.15-2.15)         | 1.56 (0.96-2.54)   | 1.40 (1.10-1.79) |
| O2-O3                  | 1.76 (1.27-2.44)   | 1.72 (1.15-2.58)         | 2.27 (1.29-3.99)   | 1.67 (1.22-2.30) |
| Year of endoscopic screening |                     |                          |                    |                |
| 2003-2007              | 1 (referent)       | 1 (referent)             | 1 (referent)       | 1 (referent)   |
| 2008-2012              | 0.45 (0.35-0.57)   | 0.58 (0.43-0.79)         | 0.37 (0.23-0.59)   | 0.60 (0.47-0.76) |
| 2013-2018              | 0.32 (0.24-0.42)   | 0.37 (0.25-0.53)         | 0.27 (0.16-0.46)   | 0.68 (0.52-0.89) |

Note: Multivariate odds ratios were estimated using a logistic regression model with all the variables entered.

Abbreviations: ADH1B, alcohol dehydrogenase-1B; ALDH2, aldehyde dehydrogenase-2; CI, confidence interval; DIULs, distinct iodine-unstained lesions; HH/CLE, hiatal hernia and/or columnar-lined esophagus; MCV, mean corpuscular volume; OR, odds ratio; SCC, squamous cell carcinoma.
On the other hand, the incidences of HH and CLE, which are indicators of gastroesophageal reflux, are increased by excessive drinking.\textsuperscript{42,43} The increment of gastric juice acidity,\textsuperscript{44} and the absence of \textit{H. pylori} infection.\textsuperscript{45} In the present population, the presence of HH/CLE $\geq 10$ mm was also positively associated with a milder degree of CAG and the absence of \textit{H. pylori} infection. Antiseptic acid reflux with an HH/CLE $\geq 10$ mm and milder or no presence of HH/CLE $\geq 10$ mm was also positively associated with a milder degree of CAG and the absence of these DIULs in combination or alone are required to clarify the difference between DIULs $\geq 5$ mm and multiple DIULs as cancer biomarkers and to assess the importance of trends in risk indicators of ESCC.

We did not observe any effects of recent alcohol consumption on the detection of DIULs. This result might be related to the homogeneity of the study population, with regard to alcohol dependence. Nationwide surveys conducted in 2003, 2008, and 2013 showed that the percentage of alcohol dependence did not change among Japanese men, but declining trends in the percentage of daily drinkers and the amount of alcohol consumed per week were reported for Japanese men.\textsuperscript{50} Changes in the drinking culture of Japan during this period may have affected the long-term drinking profiles of alcohol-dependent patients. A study examining the effects of alcohol consumption for a longer period of time could provide useful information. We cannot rule out the possibility that changes in endoscope models and the diagnostic skills of endoscopists might have influenced the reduction in the detection of DIULs. However, the detection rates of early ESCC, as well as DIULs, have markedly decreased without any changes in cancer depth during the same period,\textsuperscript{4} while endoscopic images have been improved by new models of endoscopes. Almost all the screening examinations were performed by a single endoscopist or were performed under his supervision. Another limitation was that factors such as \textit{H. pylori} infection during the entire study period, actual gastroesophageal reflux, oral-esophageal-gastric microbiota, oral hygiene, and dietary habits (including the intake of fruit and vegetables and high-temperature beverages) were not examined, and all these factors might have had considerable impacts on carcinogenesis in the esophagus.\textsuperscript{51-53}

In conclusion, the detection of DIULs and ESCC decreased between 2003 and 2018 in an alcohol-dependent population. In addition to reported determinants of ESCC, CAG and HH/CLE were associated with the risk of DIULs. Enigmatically, however, the declining trend in DIULs was not adequately explained by these factors and warrants further research.

CONFLICT OF INTEREST
The authors have no conflict of interest.

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