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Plasma insulin, C-peptide and blood glucose and the risk of gastric cancer: The Japan Public Health Center-based prospective study

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To date, the association between diabetes mellitus (DM) and gastric cancer has been controversial, including the underlying mechanism. We investigated the association between plasma diabetic biomarkers (insulin, C-peptide, and blood glucose) and gastric cancer risk. In addition, homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated. A total of 36,745 subjects aged 40–69 years in the Japan Public Health Center-based prospective study (JPHC) who returned the baseline questionnaire and provided blood samples were followed from 1990 to 2004. In the present analysis, 477 cases and 477 matched controls were used. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) for developing gastric cancer were calculated using conditional logistic regression models. Plasma insulin was positively associated with increased risk of gastric cancer; compared to tertile 1, ORs were 1.69 (95% CI = 1.11–2.59) and 2.01 (1.19–3.38) for tertiles 2 and 3, respectively (p for trend = 0.009). In men, C-peptide was also positively associated with a significant risk; corresponding ORs were 1.42 (0.85–2.38) and 1.91 (1.03–3.54), respectively (p for trend = 0.04). These findings were confirmed for blood samples from the fasting group (≥8 hr after a meal). Higher HOMA-IR was also associated with increased risk, whereas no association was observed for blood glucose. Our findings suggest that Japanese population with higher insulin and C-peptide levels derived from insulin resistance have an elevated risk of gastric cancer.

Epidemiology

Gastric cancer is the second leading cause of death and the fourth most common cancer in the world.[1] Although *Helicobacter pylori* (*H. pylori*) infection is well known as a major risk factor for gastric cancer, only some of the people infected with *H. pylori* will develop gastric cancer. Therefore, other risk factors might affect the association between *H. pylori* and gastric cancer occurrence.

Diabetes mellitus (DM) is associated with many types of cancer, including colorectal, liver, breast, and pancreatic cancer.[2] However, the association between DM and gastric cancer remains to be clarified. Some prospective studies reported that DM determined by questionnaire or medical records is positively associated with gastric cancer,[3–6] but others found a null association.[7–12] However, DM can be easily misclassified when based on self-report of disease in questionnaire survey or medical records. To overcome this problem, several studies were directly based on diabetic biomarkers, such as hemoglobin A1c (HbA1c) and blood glucose, but the associations were also inconsistent in these prospective studies.[13–16]

Key words: gastric cancer risk, plasma insulin, plasma C-peptide, plasma blood glucose, prospective study

Abbreviations: BMI: body mass index; CagA: cytotoxin associated gene A; CI: confidence interval; DM: diabetes mellitus; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β-cell function; ICD-O: international classification of diseases for oncology; IGF: insulin-like growth factor; JPHC: Japan public health center-based prospective study; OR: odds ratio; PHC: public health center; SD: standard deviation

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What's new?
The idea that diabetes mellitus may play a role in some instances of gastric carcinogenesis is intriguing but controversial. Here, a positive association was identified for gastric cancer risk and plasma insulin levels, based on investigation of plasma biomarkers in a Japanese study population. The association was evident for measures of homeostasis model assessment of insulin resistance (HOMA-IR). By contrast, no association was found for blood glucose levels. The results suggest that hyperinsulinemia derived from insulin resistance, rather than hyperglycemia, is important in gastric carcinogenesis.

Another possible candidate biomarker is insulin, which may be involved in the biological mechanisms of carcinogenesis that underlie the association between DM and gastric cancer. To date, several *in vivo* and *in vitro* studies have reported a positive association between insulin and carcinogenesis including gastric mucosa.[17,18] To our knowledge, no prospective study has evaluated the association between insulin and the risk of gastric cancer.

In this study, we investigated the association between plasma insulin, C-peptide, and blood glucose and gastric cancer risk in a case-control study nested within a large-scale population-based study. C-peptide is a metabolic product of insulin and is more stable than insulin in blood. In addition, we calculated homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) to evaluate the extent of insulin resistance and pancreatic β-cell function,[19] respectively.

Material and Methods

Study population

The Japan Public Health Center-based prospective study (JPHC) was established in 1990 for cohort I (subject age range 40–59 years) and in 1993 for cohort II (40–69 years), as described previously.[20] The JPHC consisted of 11 public health centers (PHCs) in Japan and included 140,420 subjects (68,722 men and 71,698 women). The subjects from one PHC (Tokyo) in cohort I were excluded from this study because the data on cancer incidence were not available. In addition, one subgroup of cohort II (Osaka) was excluded because the selection of subjects differed from that of other cohort subjects, which left 123,576 subjects (61,009 men and 62,567 women). This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In the baseline survey, a self-administered questionnaire was used in each cohort. The study subjects were asked about various lifestyle factors, such as sociodemographic characteristics, personal medical history, family history, smoking and drinking habits, dietary habits and physical activity. A total of 99,808 subjects (47,525 men and 52,283 women) responded (response rate: 80.8%).

We asked each subject to provide a 10-ml blood sample at the time of the health checkup. After exclusion of subjects who self-reported cancer at baseline ($n = 2136$), who were non-Japanese ($n = 18$), and who did not live in the area at the baseline ($n = 11$), 97,644 subjects (46,803 men and 50,841 women) remained eligible. (One subject both self-reported cancer at baseline and was non-Japanese.) Among the eligible subjects, 36,745 subjects (13,467 men and 23,278 women) provided blood samples at baseline. Plasma levels of blood glucose were measured at each PHC area at the time of the baseline health check-up and the values were used for the present analysis. One PHC (Niigata) in cohort II and two PHCs (Akita and Iwate) in cohort I did not routinely measure glucose ($n = 174$). According to the Osaka Medical Center for Health Science and Promotion, the accuracy of plasma blood glucose measurements in all the laboratories was found to be satisfactory.[21] The plasma and buffy coat were divided into four tubes, each holding 1.0 ml (three tubes for plasma and one for the buffy coat), and then preserved at −80°C until analysis.

The blood samples were collected from 1990 to 1992 in cohort I and from 1993 to 1995 in cohort II. Following the standard protocol, we requested that subjects avoid having a meal after 21:00 on the day before the health checkup, and recorded the approximate last time of caloric intake, including a meal and/or drinking.

Follow-up

Subjects were observed from 1 January 1990 to 31 December 2004 for cohort I and from 1 January 1993 to 31 December 2004 for cohort II. Residence status, survival, and death were identified annually through residential registries in each PHC area. In Japan, residence and death registration are required by law, and the registries are believed to be complete. Among the 36,745 subjects, 1,423 (3.9%) moved outside the study area, 1,610 (4.4%) died, and 11 (0.03%) were lost to follow-up during the study period.

Cancer registry for the JPHC

Incidence data on gastric cancer cases were collected for the JPHC cancer registry from two sources: local major hospitals and population-based cancer registries (usually prefecture-wide). Death certificate information was also used. In our cancer registry system, information for 7.6% of gastric cancer cases was based on the case first identified *via* a death certificate and 2.1% were registered based on information from the death certificate alone.

Selection of cases and controls

Over the entire study period from 1990 to 2004, 1681 new gastric cancer cases with a histologically proven diagnosis

Table 1. Baseline characteristics of cases and controls

Characteristics	Cases	Controls	*p* value[1]
N	477	477	
Age, mean (SD)	57.2 (7.19)	57.2 (7.21)	Matching value
Men (%)	319 (66.9)	319 (66.9)	Matching value
Smoking status			
Never smoker (%)	218 (45.7)	237 (49.7)	
Past smoker (%)	88 (18.5)	93 (19.5)	
Current ≤20 cigarettes/day (%)	132 (27.7)	106 (22.2)	
Current ≥21 cigarettes/day (%)	39 (8.1)	41 (8.6)	0.28
Alcohol consumption			
Never or occasional (%)	229 (48.0)	236 (49.5)	
≥1 day, <300 g/week (%)	185 (38.8)	194 (40.7)	
≥1 day, ≥300 g/week (%)	63 (13.2)	47 (9.8)	0.27
BMI (kg m^{-2})[2]			
BMI < 22 (%)	169 (35.7)	158 (33.3)	
22 ≤ BMI < 25 (%)	207 (43.8)	198 (41.7)	
25 ≤ BMI (%)	97 (20.5)	119 (25.0)	0.25
Family history of gastric cancer (%)	58 (12.2)	39 (8.2)	0.04
Past history of DM (%)	44 (9.2)	21 (4.4)	0.003
Drug treatment for DM (%)	15 (3.1)	8 (1.7)	0.14
Helicobacter pylori positive (%)[3]	449 (94.1)	357 (74.8)	<0.001
CagA positive (%)	359 (75.3)	335 (70.2)	0.08
Atrophy (%)[4]	390 (81.8)	278 (58.3)	<0.001

[1]Based on chi-square test or Student's *t* test.
[2]Subjects for whom we were unable to calculate body mass index due to missing height or weight data (four cases and two controls) were deleted.
[3]Based on immunoglobulin G antibody.
[4]Atrophy: positive if pepsinogen I ≤ 70 ng ml^{-1} and pepsinogen I/pepsinogen II ratio ≤3.
Abbreviations: BMI: body mass index; CagA: cytotoxin associated gene A; DM: diabetes mellitus; SD: standard deviation.

were observed in the two cohorts. Among these cases, blood samples and questionnaire responses at baseline had been obtained from 512 cases. The anatomic subsite of each case was coded on the basis of the International Classification of Diseases for Oncology (ICD-O), 3rd edition.[22] Tumor located in the upper third of the stomach was referred to as proximal gastric cancer (cardia subsite) (ICD-O code C16.0 and 16.1), and that in the lower portion of the stomach was classified as distal gastric cancer (non-cardia subsite) (ICD-O code C16.2–16.7). The remaining cases were tumors that could not be classified because of overlapping lesions (ICD-O code C16.8) or no information (ICD-O code C16.9). The subdivisions by histological type were based on the Lauren classification.[23] For each case, we selected one control subject from those who were not diagnosed with gastric cancer during the follow-up period when the case was diagnosed. We matched case and control for gender, age (±3 years), study area, fasting time at blood donation (±5 hr), and blood donation date (±2 months). Among the 512 new gastric cancer cases, 1 case was excluded due to a technical error in the measurement of *H. pylori* and 34 cases were excluded due to no volume left for the present measurement. The final analysis included 477 matched sets of cases and controls.

Laboratory assays for insulin and C-peptide

Plasma levels of insulin and C-peptide were measured at GeneticLab, Hokkaido, Japan. All laboratory personnel were blinded about case and control status. Plasma diabetic biomarkers were simultaneously assayed using a Human Endocrine Milliplex Kit (#HEND-65K; Millipore Company, 6 Research Park Drive, St. Charles, MO). The kit used polystyrene bead–based assays to measure the markers in 25-μl samples across panels. Based on the measurement of eight median fluorescent intensities, a standard curve of the biomarker was used to convert optical density values into concentrations, with limits of assay detection of 5.8 pg ml^{-1} (1 pmol l^{-1}) for insulin and 3.6 pg ml^{-1} (1 pmol l^{-1}) for C-peptide. Using the curve-fit measurements for each standard, technicians also estimated coefficients of variation, which were calculated as the ratio of the observed and expected concentrations. The average coefficients of variation for plasma levels of insulin and C-peptide were 7.2 and 4.2%, respectively. Some plasma samples could not be measured because of insufficient volume: 27 for insulin and 2 for C-peptide.

Statistical analysis

Tertiles of plasma diabetic biomarkers and HOMA-β were based on levels in control subjects. The chi-square test and Student's *t* test were used to compare background characteristics between cases and controls. Matched odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using conditional logistic regression models. OR1 was matched for age (±3 years), gender, PHC area, blood donation date (±2 months), and fasting time at blood donation (±5 hr). OR2 was calculated by multivariate conditional logistic regression analysis adjusting for potential confounding factors such as smoking status, alcohol consumption, total calorie intake, salt intake, body mass index (BMI), family history of gastric cancer, *H. pylori* infection status, and atrophy. OR3 was further adjusted for past history of DM and drug treatment for DM.

Smoking status was divided into four groups: never smoker, past smoker, current smoker with ≤20 cigarettes per day, and current smoker with ≥21 cigarettes per day. Alcohol consumption was divided into four groups: never drinker, occasional drinker, current drinker who intakes <300 g of ethanol per week, and current drinker who intakes ≥300 g of

Table 2. ORs and 95% CIs for the association between plasma levels of diabetic biomarkers and gastric cancer risk

		Cases (*n*)/ controls (*n*)	OR1 (95%CI)[1]	OR2 (95% CI)[2]	OR3 (95% CI)[3]
Insulin (pg ml^{-1})	Tertile 1 (10.7–228.7)	137/152	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (233.1–468.7)	163/153	1.25 (0.87–1.80)	1.63 (1.08–2.47)	1.68 (1.10–2.56)
	Tertile 3 (471.0–7933.3)	157/152	1.36 (0.88–2.11)	1.91 (1.15–3.18)	2.03 (1.21–3.41)
	p for trend		0.17	0.01	0.007
C-peptide (pg ml^{-1})	Tertile 1 (130.5–653.6)	160/158	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (659.7–1292.8)	160/159	0.99 (0.70–1.40)	1.15 (0.77–1.71)	1.15 (0.77–1.72)
	Tertile 3 (1303.0–8739.4)	155/158	1.02 (0.68–1.55)	1.31 (0.82–2.11)	1.30 (0.81–2.10)
	p for trend		0.92	0.26	0.28
Blood glucose (mg dl^{-1})	Tertile 1 (72.0–92.0)	138/124	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (93.0–106.0)	114/124	0.81 (0.55–1.18)	1.01 (0.66–1.55)	0.98 (0.63–1.50)
	Tertile 3 (107.0–406.0)	121/125	0.85 (0.57–1.29)	0.96 (0.61–1.53)	0.84 (0.52–1.36)
	p for trend		0.41	0.88	0.50

[1]Matched for age (±3 years), gender, public health center area, blood donation date (±2 months), and fasting time at blood donation (±5 hr).
[2]Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.
[3]Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.
Abbreviations: CI: confidence interval; OR: odds ratio.

ethanol per week. Total calorie and salt intakes were treated as continuous variables. BMI was divided into three classes: BMI < 22 kg m^{-2}, $22 \leq$ BMI < 25, and $25 \leq$ BMI. Subjects who were missing value for BMI ($n = 6$), total calorie ($n = 1$), and salt intakes ($n = 1$) were excluded when adjusting for these confounding factors. Family history of gastric cancer was considered positive if at least one parent or sibling had gastric cancer. The *H. pylori* infection status was regarded as positive if subjects had either *H. pylori* antibody ≥ 10 U ml^{-1} or cytotoxin associated gene A (CagA) antibody >10. Atrophy was regarded as positive if pepsinogen I was ≤ 70 ng ml^{-1} and the pepsinogen I/pepsinogen II ratio was ≤ 3.[24] Because we do not have any data from upper gastrointestinal endoscopies and biopsies, the pepsinogen data were used. Urita et al. reported that the pepsinogen I/pepsinogen II ratio ≤ 3 identified gastric atrophy with a sensitivity of 71.7% and a specificity of 66.7%.[25] We believe that the pepsinogen data could explain the level of atrophy, to some extent, if added to the model. Past history of DM and drug treatment for DM were considered positive if subjects were diagnosed with DM before and used a diabetic drug at the time of the baseline survey, respectively. Stratified analysis based on fasting status (≥ 8 hr or <8 hr after a meal) was also conducted for each plasma diabetic biomarker. Furthermore, for the subjects who were in the fasting group (≥ 8 hr after a meal) at blood donation and not under drug treatment for DM, we calculated HOMA-IR [fasting plasma insulin level (μU ml^{-1}) × fasting plasma glucose level (mg dl^{-1})/405] and HOMA-β [360 × fasting plasma insulin level (μU ml^{-1})/fasting plasma glucose level (mg dl^{-1}) − 63].[19] HOMA-IR ≥ 1.73 was defined as the presence of insulin resistance.[26] According to the manufacturer of the insulin measuring kit (Millipore), conversion of insulin units was based on the human insulin international reference preparation of WHO (1 μIU ml^{-1} = 35 pg ml^{-1}).

Reported *p* values are two-sided, and $p < 0.05$ was defined as statistically significant. All statistical analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC).

Results

Baseline characteristics of cases and controls are shown in Table 1. Family history of gastric cancer, past history of DM, *H. pylori* positivity, and atrophy were significantly more frequent among cases compared to controls. The distributions of other factors were similar in cases and controls. At baseline, 9.2% of cases and 4.4% of controls had past history of DM, and 3.1% of cases and 1.7% of controls had received drug treatment for DM.

Table 2 shows ORs and 95% CIs for the associations between plasma levels of diabetic biomarkers and gastric cancer risk using conditional logistic regression models. We found that plasma insulin was dose-dependently associated with an increased risk of gastric cancer. Compared to tertile 1, OR2 (adjusted for smoking, alcohol consumption, BMI, total calories, salt intake, family history of gastric cancer, *H. pylori* infection status, and atrophy) for tertiles 2 and 3 was 1.63 (95% CI = 1.08–2.47) and 1.91 (1.15–3.18), respectively (*p* for trend 0.01). When further adjusted for past history of DM and drug treatment for DM, corresponding values for OR3 were 1.68 (1.10–2.56) and 2.03 (1.21–3.41), respectively (*p* for trend 0.007). We found no association between the other diabetic biomarkers and risk of gastric cancer.

In Table 3, the associations between plasma levels of diabetic biomarkers and gastric cancer risk are shown for men

Table 3. ORs and 95% CIs for the association between plasma levels of diabetic biomarkers and gastric cancer risk in men and women

		Cases (*n*)/ controls (*n*)	OR1 (95% CI)[1]	OR2 (95% CI)[2]	OR3 (95% CI)[3]
Men					
Insulin (pg ml^{-1})	Tertile 1 (10.7–224.3)	92/102	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (226.4–491.0)	108/103	1.29 (0.82–2.03)	1.76 (1.00–3.09)	1.75 (0.99–3.10)
	Tertile 3 (495.9–7933.3)	107/102	1.50 (0.87–2.60)	2.43 (1.23–4.78)	2.49 (1.25–4.96)
	p for trend		0.15	0.01	0.01
C-peptide (pg ml^{-1})	Tertile 1 (130.5–643.1)	95/106	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (644.2–1380.9)	111/106	1.25 (0.82–1.90)	1.39 (0.83–2.30)	1.43 (0.86–2.40)
	Tertile 3 (1388.3–8739.4)	112/106	1.42 (0.85–2.38)	1.90 (1.04–3.48)	1.96 (1.06–3.64)
	p for trend		0.18	0.04	0.03
Blood glucose (mg dl^{-1})	Tertile 1 (73.0–94.0)	91/87	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (95.0–108.0)	70/81	0.81 (0.51–1.29)	0.91 (0.53–1.57)	0.92 (0.54–1.59)
	Tertile 3 (109.0–406.0)	89/82	1.07 (0.66–1.74)	1.18 (0.67–2.08)	1.02 (0.57–1.83)
	p for trend		0.85	0.59	0.98
Women					
Insulin (pg ml^{-1})	Tertile 1 (41.1–238.4)	49/50	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (239.8–429.1)	54/50	1.05 (0.57–1.93)	1.44 (0.71–2.94)	1.61 (0.77–3.37)
	Tertile 3 (430.1–5237.4)	47/50	0.91 (0.45–1.84)	1.08 (0.48–2.46)	1.27 (0.54–3.00)
	p for trend		0.79	0.81	0.56
C-peptide (pg ml^{-1})	Tertile 1 (158.2–679.1)	69/52	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (685.7–1181.6)	43/53	0.44 (0.22–0.88)	0.58 (0.27–1.26)	0.54 (0.25–1.20)
	Tertile 3 (1183.2–3496.9)	45/52	0.46 (0.22–0.97)	0.59 (0.25–1.39)	0.58 (0.25–1.38)
	p for trend		0.04	0.23	0.23
Blood glucose (mg dl^{-1})	Tertile 1 (72.0–90.0)	50/41	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (91.0–103.0)	37/42	0.69 (0.36–1.35)	0.89 (0.41–1.97)	0.88 (0.39–1.98)
	Tertile 3 (104.0–235.0)	36/40	0.69 (0.32–1.51)	0.59 (0.22–1.57)	0.48 (0.17–1.33)
	p for trend		0.29	0.32	0.19

[1]Matched for age (±3 years), public health center area, blood donation date (±2 months), and fasting time at blood donation (±5 hr).
[2]Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.
[3]Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.
Abbreviations: CI: confidence interval; OR: odds ratio.

and women separately. In men, besides insulin, plasma C-peptide was also dose-dependently associated with gastric cancer risk; OR2 was 1.39 (0.83–2.30) and 1.90 (1.04–3.48) for tertiles 2 and 3, respectively (*p* for trend 0.04). Corresponding values for OR3 were 1.43 (0.86–2.40) and 1.96 (1.06–3.64), respectively (*p* for trend 0.03). In women, plasma C-peptide was inversely associated with gastric cancer risk (OR1), but it lost statistical significance after further adjustment (OR2 and OR3).

Participants who provided blood samples more than 8 hr after a meal were defined as the fasting group. Because plasma insulin and C-peptide showed positive associations with gastric cancer (Tables 2 and 3), further stratified analysis by fasting status (≥8 hr and <8 hr after a meal) was performed for these biomarkers, as well as HOMA-IR and HOMA-β. After excluding pairs with different fasting status, conditional logistic regression analysis was conducted (Table 4). The levels of these biomarkers differed by fasting status. We found that higher levels of plasma insulin and C-peptide were marginally associated with gastric cancer risk in the fasting group (≥8 hr after a meal). For the non-fasting group (<8 hr after a meal), whose biomarker levels may be strongly influenced by the meal, a weakly increased risk was also observed, but not significantly so. Moreover, a higher HOMA-IR was associated with increased risk of gastric cancer; OR2 for HOMA-IR ≥1.73 was 1.88 (1.03–3.45) compared to HOMA-IR <1.73. Corresponding values for OR3 were 1.97 (1.07–3.65). Higher HOMA-β also showed a trend toward a positive association.

We conducted stratified analyses by alcohol consumption, smoking status, menopausal status (menopausal or not menopausal), and atrophy, and no differences according to such

Table 4. ORs and 95% CIs by fasting status for the association between insulin, C-peptide, HOMA-IR, and HOMA-β and gastric cancer risk

		Cases (*n*)/ controls (*n*)	OR1 (95%CI)[1]	OR2 (95%CI)[2]	OR3 (95%CI)[3]
Non-fasting group[4]					
Insulin (pg ml^{-1})	Tertile 1 (92.3–366.5)	92/86	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (367.4–621.1)	81/87	0.84 (0.51–1.36)	1.07 (0.58–1.98)	1.03 (0.56–1.91)
	Tertile 3 (628.1–7933.3)	86/86	0.94 (0.56–1.59)	1.26 (0.66–2.42)	1.21 (0.63–2.32)
	p for trend		0.84	0.47	0.56
C-peptide (pg ml^{-1})	Tertile 1 (140.4–1012.2)	93/89	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (1022.3–1755.5)	87/89	0.94 (0.57–1.54)	1.29 (0.72–2.30)	1.26 (0.70–2.27)
	Tertile 3 (1762.0–8739.4)	87/89	0.96 (0.56–1.64)	1.52 (0.79–2.93)	1.54 (0.79–2.98)
	p for trend		0.89	0.21	0.20
Fasting group[4]					
Insulin (pg ml^{-1})	Tertile 1 (10.7–179.5)	51/62	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (180.3–283.3)	72/63	1.42 (0.84–2.41)	1.62 (0.89–2.93)	1.58 (0.87–2.88)
	Tertile 3 (286.0–4457.3)	65/63	1.35 (0.76–2.40)	1.84 (0.93–3.63)	1.89 (0.95–3.77)
	p for trend		0.31	0.08	0.07
C-peptide (pg ml^{-1})	Tertile 1 (130.5–493.6)	54/65	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (497.5–755.4)	78/66	1.39 (0.86–2.26)	1.68 (0.95–2.97)	1.80 (1.00–3.24)
	Tertile 3 (776.0–2717.4)	65/66	1.23 (0.72–2.08)	1.80 (0.92–3.53)	1.76 (0.89–3.47)
	p for trend		0.46	0.09	0.10
HOMA-IR[5]	<1.73	96/104	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	≥1.73	60/52	1.29 (0.79–2.11)	1.88 (1.03–3.45)	1.97 (1.07–3.65)
HOMA-β (%)[5]	Tertile 1 (17.6–52.7)	41/52	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (53.3–89.0)	58/52	1.49 (0.82–2.69)	1.34 (0.67–2.67)	1.45 (0.71–2.93)
	Tertile 3 (89.3–1580.9)	57/52	1.47 (0.81–2.66)	1.60 (0.81–3.14)	1.94 (0.94–4.03)
	p for trend		0.23	0.17	0.08

[1]Matched for age (±3 years), gender, public health center area, and blood donation date (±2 months).
[2]Adjusted for smoking, alcohol consumption, body mass index, total calories, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, and atrophy.
[3]Further adjusted for past history of diabetes mellitus and drug treatment for diabetes mellitus.
[4]Fasting group: ≥8 hr after a meal; Non-fasting group: <8 hr after a meal.
[5]Subjects under drug treatment for diabetes mellitus were excluded, and OR3 was further adjusted for past history of diabetes mellitus only.
Abbreviations: HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β-cell function; CI: confidence interval; OR: odds ratio.

stratification were observed. Higher insulin and C-peptide levels were positively associated with the distal subsite and intestinal type of gastric cancer risk, but not significantly so. In addition, the cardia subsite and diffuse type of gastric cancer also showed a trend toward a positive association with insulin, but not with C-peptide, possibly due to the small number of subjects (data not shown). When we excluded the subjects with a past history of DM and drug treatment for DM, similar associations were observed between plasma insulin and C-peptide and gastric cancer risk. Higher HOMA-IR and HOMA-β values also showed similar associations when subjects with past history of DM were excluded (data not shown). Finally, when we excluded the subjects who developed gastric cancer within 2 years of blood donation and their matched controls, similar associations were observed (data not shown).

Discussion

In this case-control study nested within a large-scale population-based study, we observed an increased risk of gastric cancer according to higher insulin levels, C-peptide levels, and HOMA-IR, independent of several confounding factors. The positive association was also observed when excluding subjects who had past history of DM and drug treatment for DM. In contrast, plasma levels of blood glucose were not associated with gastric cancer risk. No association was observed for any of the diabetic biomarkers in women.

Several postulated DM-related mechanisms of carcinogenesis, including hyperglycemia itself and/or decreased bioactivity of insulin such as hyperinsulinemia or insulin resistance, have been controversial.[27,28] A meta-analysis of several prospective studies reported that not only higher levels of insulin and C-peptide but also higher levels of blood glucose

significantly increased the risk of pancreatic and colorectal cancers.[29] But this meta-analysis had a critical limitation, in that few studies took fasting status into account. In more recent reports of large population-based nested case-control studies of pancreatic and colorectal cancer, fasting group (≥8 hr after a meal) was considered. For the risk of pancreatic cancer, when HbA1c and insulin were adjusted, only a higher level of plasma proinsulin was found to increase the risk, whereas the proinsulin/insulin ratio, a marker of pancreatic β-cell function, was not.[30] For the risk of colorectal cancer, higher insulin level and HOMA-IR were associated with an increased risk, whereas no association was observed for blood glucose.[31] Therefore, the authors concluded that their results did not support the hypothesis that hyperglycemia is causally associated with increased risk of pancreatic and colorectal cancers. We observed that higher levels of insulin and C-peptide significantly increase the risk of gastric cancer, not blood glucose levels. This may suggest the importance of hyperinsulinemia, rather than hyperglycemia, in gastric carcinogenesis as well as other cancer sites, such as pancreatic and colorectal cancer.

Insulin is a well-known key regulator of carcinogenesis, including gastric cancer.[17,18,32] Insulin can enhance insulin-like growth factor (IGF)−1 bioavailability by inhibiting the production of IGF-binding proteins.[18,32] Insulin and bioavailable IGF-1 signal transduction occurs through insulin, IGF-1, and hybrid receptors in the cell membrane.[18] Inhibition of apoptosis and stimulation of cellular proliferation and carcinogenesis occurs because of the several downstream pathways activated by these receptors. The binding of insulin or bioavailable IGF-1 to the receptors activates phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) and Ras/MAPK (mitogen-activated protein kinase) pathways.[18]

In our study, the positive associations between plasma insulin and C-peptide levels and gastric cancer occurrence were clearly observed in men, but not in women. One possible explanation is hormonal differences. A recent meta-analysis showed that women with longer exposure to estrogen by either ovarian (fertility) or exogenous origin (hormone replacement therapy) may be protected from gastric cancer,[33] and that the body mass of postmenopausal women correlates with blood estrogen levels.[34] The possible protective effect of estrogen might mask the risk of developing gastric cancer in women, although the analysis stratified by menopausal status (menopausal or not menopausal) did not show a clear difference between the two. Another explanation is that alcohol consumption[35] and smoking[36] may determine insulin resistance and hyperinsulinemia thereby resulting in gastric carcinogenesis. In our study, most alcohol drinkers and smokers were male. However, additional analysis did not show any clear interaction between smoking status or alcohol consumption and diabetic biomarkers.

In the fasting group (≥8 hr after a meal), we analyzed not only plasma insulin and C-peptide levels, but also HOMA-IR and HOMA-β. By calculating HOMA, we can estimate the background of hyperinsulinemia at fasting group such as insulin resistance (HOMA-IR) and/or greater functioning of pancreatic β-cell function (HOMA-β). We found that higher HOMA-IR was positively associated with gastric cancer risk. Therefore, our findings suggest that insulin resistance is the main mechanism underlying the positive association between hyperinsulinemia and gastric cancer risk. HOMA-β also showed a marginal association. One previous study showed an increasing pancreatic β-cell volume to compensate for insulin resistance,[37] which may result in increased β-cell function. A possible explanation for insulin resistance leading to hyperinsulinemia may be that it is a consequence of *H. pylori* infection. According to a recent systematic review, a positive trend toward an association between *H. pylori* infection and insulin resistance was found.[38] Several mechanisms underlying the relationship between *H. pylori* infection and insulin resistance suggest that reactive oxygen species, proatherogenic substances, and inflammatory substances are released by *H. pylori* infection. *H. pylori* infection also promotes the activation/aggregation of platelets and apoptosis.[39]

This is the first population-based prospective study to indicate a positive association between higher levels of insulin and C-peptide and gastric cancer risk. Based on the study design, the blood samples were collected before subjects were diagnosed with gastric cancer, which enabled us to investigate the factors associated with a subsequent risk of gastric cancer incidence. In addition, we have robust data on other factors including fasting status, history of DM, drug treatment for DM, lifestyle factors, atrophy, CagA, and *H. pylori* infection.

Our study did have some limitations. First, among the 97,644 eligible subjects who responded to a self-administered questionnaire in this study, only 36,745 (37.6%) subjects provided a blood sample. Those subjects who participated in the health checkup survey had a more favorable lifestyle, such as less smoking and alcohol consumption, as compared to those who did not participate. Therefore, generalizing the findings of this study to a large population needs to be performed carefully, as described previously.[40] Second, these diabetic biomarkers were measured only once at the baseline. We do not have information regarding the onset of DM in those with high-level diabetic biomarkers, so we cannot speculate regarding the length of suffering attributable to DM. Moreover, given that the follow-up of the subjects lasted for many years, it is possible that these levels might have changed over the course of the years. However, this is not different between cases and controls and likely would have led to underestimation of the results. Third, it is difficult to completely exclude undiagnosed gastric cancer at the baseline survey because past history of gastric cancer was based on self-administered questionnaire. However, when we excluded those subjects who developed gastric cancer within 2 years of blood donation based on the cancer registry, similar associations were obtained. Fourth, with regard to asking past history of DM, we did not distinguish between type 1 and type 2 DM in the questionnaire. However, because type 1 DM is far less frequent then type 2 DM, especially in the adult population, it would be

reasonable to suppose that most of the subjects had type 2 DM. Fifth, we did not have data regarding HbA1c or adequate samples to measure HbA1c. HbA1c levels reflect mean blood glucose over the preceding 3 months. Thus, it is possible that we might have missed subjects who were pre-diabetic or subjects with optimal blood glucose control. Sixth, the proportion of the subjects in the non-fasting group was much higher than that in the fasting group, which may have an effect on the validity of our observations. Therefore, caution should be used when interpreting the results. Finally, the number of subjects may not have been sufficient to identify the association in some anatomic sites or histological types. Therefore, additional large prospective studies are needed to confirm the association in cardia subsite and diffuse type gastric cancer.

In conclusion, our findings suggest that Japanese population with higher insulin and C-peptide levels derived from insulin resistance have an elevated risk of gastric cancer.

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Appendix

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Genetic polymorphisms of *ADH1B*, *ADH1C* and *ALDH2*, alcohol consumption, and the risk of gastric cancer: the Japan Public Health Center-based prospective study

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Abstract

The association between alcohol consumption, genetic polymorphisms of alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) and gastric cancer risk is not completely understood. We investigated the association between *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms, alcohol consumption and the risk of gastric cancer among Japanese subjects in a population-based, nested, case–control study (1990–2004). Among 36 745 subjects who answered the baseline questionnaire and provided blood samples, 457 new gastric cancer cases matched to 457 controls were used in the analysis. The odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated using logistic regression models. No association was observed between alcohol consumption, *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms and gastric cancer risk. However, considering gene–environmental interaction, *ADH1C* G allele carriers who drink ≥150 g/week of ethanol had a 2.5-fold increased risk of gastric cancer (OR = 2.54, 95% CI = 1.05–6.17) relative to AA genotype carriers who drink 0 to <150 g/week (*P* for interaction = 0.02). *ALDH2* A allele carriers who drink ≥150 g/week also had an increased risk (OR = 2.08, 95% CI = 1.05–4.12) relative to GG genotype carriers who drink 0 to < 150 g/week (*P* for interaction = 0.08). To find the relation between alcohol consumption and gastric cancer risk, it is important to consider both alcohol consumption level and *ADH1C* and *ALDH2* polymorphisms.

Introduction

Alcohol consumption is a strong risk factor for some cancers of the head and neck, liver, breast and colon and rectum (1). However, based on many epidemiological studies, the association between alcohol consumption and gastric cancer risk was reported as inconsistent by the World Cancer Research Fund/ American Institute for Cancer Research (2).

In general, the metabolism of ethanol (alcohol) by alcohol dehydrogenases (ADH) is converted into the generation of acetaldehyde, and acetaldehyde is oxidized into nontoxic acetate by aldehyde dehydrogenases (ALDH (3)). Among all classes of ADH and ALDH isoenzymes, ADH1B, ADH1C and ALDH2 are the main ethanol-metabolizing enzymes (4,5). It has been suggested that

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Abbreviations

ADH	alcohol dehydrogenases
ALDH	aldehyde dehydrogenases
BMI	body mass index
CagA	cytotoxin-associated gene A
CI	confidence interval
DM	diabetes mellitus
DR	dietary records
FFQ	food frequency questionnaire
ICD-O	International Classification of Diseases for Oncology
JPHC study	Japan Public Health Center-based prospective study
OR	odds ratio
PHC	public health center.

the metabolism of ethanol leads to accumulation of acetaldehyde (acetaldehyde associated with alcoholic beverages) that is toxic and classified as a group 1 carcinogen in humans by the International Agency for Research on Cancer (IARC (6)). Accumulation of acetaldehyde differs according to functional enzymatic *ADH1B*, *ADH1C* and *ALDH2* genetic polymorphisms. In previous studies, active *ADH1B* allele metabolizes ethanol into acetaldehyde ~40 times more than inactive allele, and active *ADH1C* allele metabolizes ~2.5 times more than inactive allele (5). Furthermore, light drinkers with inactive homozygote *ALDH2* genotype and with heterozygote genotype have 18 times and 5 times higher, respectively, average peaks of acetaldehyde concentrations in blood than moderate drinkers with active homozygote genotypes (7). Therefore, it is important to consider alcohol consumption level and functional genetic polymorphisms of ethanol-metabolizing enzymes to clarify the association between alcohol consumption and gastric cancer risk.

The genotype frequencies of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms differ according to race. The genotype frequencies of *ADH1B* and *ALDH2* polymorphisms are unevenly distributed in Caucasians, but not in Asians (8). Thus, we suggest that it is necessary to evaluate the association of *ADH1B* and *ALDH2* polymorphisms in Asians. In contrast, the genotype frequencies of the *ADH1C* polymorphism are unevenly distributed in Asians, but not in Caucasians (8). However, this polymorphism is also an important gene in alcohol metabolism, and there is no published study regarding the association between the *ADH1C* polymorphism and gastric cancer risk in Asians.

In our study, we selected genetic polymorphisms *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671), which are functionally established single nucleotide polymorphisms, and aimed to clarify the association between these genetic polymorphisms, alcohol consumption and gastric cancer risk in a large-scale Japanese population-based study. Our hypothesis was that drinkers with inactive *ADH1B* and *ADH1C* G alleles would have an increased risk for gastric cancer compared with those with active A alleles. Because inactive allele carriers cannot metabolize ethanol into acetaldehyde, they are less prone to the effects of acetaldehyde such as nausea, increased heart rate and flushing (9). International Agency for Research on Cancer classifies ethanol in alcoholic beverages as a group 1 carcinogen in humans, the same classification as acetaldehyde (6). In addition, drinkers with inactive *ALDH2* A alleles would be at increased risk compared with those with active G alleles because inactive allele carriers cannot oxidize acetaldehyde.

Materials and methods

Study population

The Japan Public Health Center-based prospective study (JPHC study) was launched in 1990 for cohort I (subject age range, 40–59 years) and in 1993 for cohort II (subject age range, 40–69 years) and investigated cancer, cardiovascular disease and other lifestyle-related diseases (10). The JPHC study consisted of 11 public health centers (PHCs) throughout Japan with a total of 140 420 subjects (68 722 men and 71 698 women). Among study subjects, those who registered at two PHC areas (Tokyo and Osaka) were excluded from this study because data regarding cancer incidence was not available or selection of subjects was defined differently from that of other cohort subjects. A population-based cohort of 123 576 subjects (61 009 men and 62 567 women) was established. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In the baseline survey, the study subjects were asked to reply to a self-administered questionnaire about various lifestyle factors, such as sociodemographic characteristics, personal medical history, family history, smoking and drinking habits, dietary habits and physical activity. A total of 99 808 subjects (47 525 men and 52 283 women) responded, giving a response rate of 80.8%.

We excluded subjects who self-reported cancer at baseline (n = 2136), who were not Japanese (n = 18) and who did not live in the area at the baseline (n = 11), which left 97 644 eligible subjects (46 803 men and 50 841 women). One subject reported having cancer at baseline and was also not Japanese. Among the eligible subjects, 36 745 subjects (13 467 men and 23 278 women) provided a 10-ml blood sample at the time of the health check-up conducted by each PHC area. These blood samples were stored at −80°C until analysis. Blood samples were collected from 1990 to 1992 for cohort I and from 1993 to 1995 for cohort II. Following the standard protocol, subjects were asked to avoid having a meal after 21:00 hours on the day before the health check-up and they recorded the last time of caloric intake (including a meal and/or drinking).

Follow-up and cancer registry for JPHC Study

Subjects were observed until 31 December 2004. In Japan, residence and death registration are required by law, and residence status, survival and death were identified annually through residential registries in each area. Among the 36 745 subjects, 3.9% moved outside the study area, 4.4% died and 0.03% were lost to follow-up during the study period, which left 33 701 subjects.

Incidence data regarding gastric cancer cases were identified from two major sources: local major hospitals in the study area and population-based cancer registries. Death certificate information was also used as an information source. In our cancer registry system, 7.6% of gastric cancer cases were based on information first notified via death certificate and 2.1% were registered based on information from the death certificate alone.

Selection of cases and controls

The anatomic site of each case was coded according to the International Classification of Diseases for Oncology (ICD-O), 3rd edition, codes C16.0–16.9 (11). A tumor located in the upper third of the stomach was classified as proximal gastric cancer 'cardia site' (ICD-O code C16.0–16.1), and that in the lower position of the stomach was classified as distal gastric cancer 'noncardia site' (ICD-O code C16.2–16.7). The other cases were tumors that could not be classified because of overlapping lesions (ICD-O code C16.8) or no information (ICD-O code C16.9). The subdivisions by histological type were based on classification derived by Lauren (12). For each case, we selected one control subject who had no history of gastric cancer when the case was diagnosed. Each control was matched to the case for age (±3 years), sex, PHC area, fasting time at blood donation (±5 h) and blood donation date (±2 months). Among 1681 cases diagnosed histologically and registered in cohort I or cohort II (study period from 1990 to 2004), 512 cases replied to a self-administered questionnaire and provided blood. Furthermore, among the 512 new gastric cancer cases, one case was excluded because of a technical error in the measurement of *Helicobacter pylori* (*H.pylori*) and 45 cases for one PHC area in Osaka were excluded because buffy coat was not available. Another nine cases were excluded because of an inadequate concentration of buffy coat for DNA extraction. The final analysis included 457 matched sets of cases and controls. A flowchart of the study subjects is presented in Figure 1.

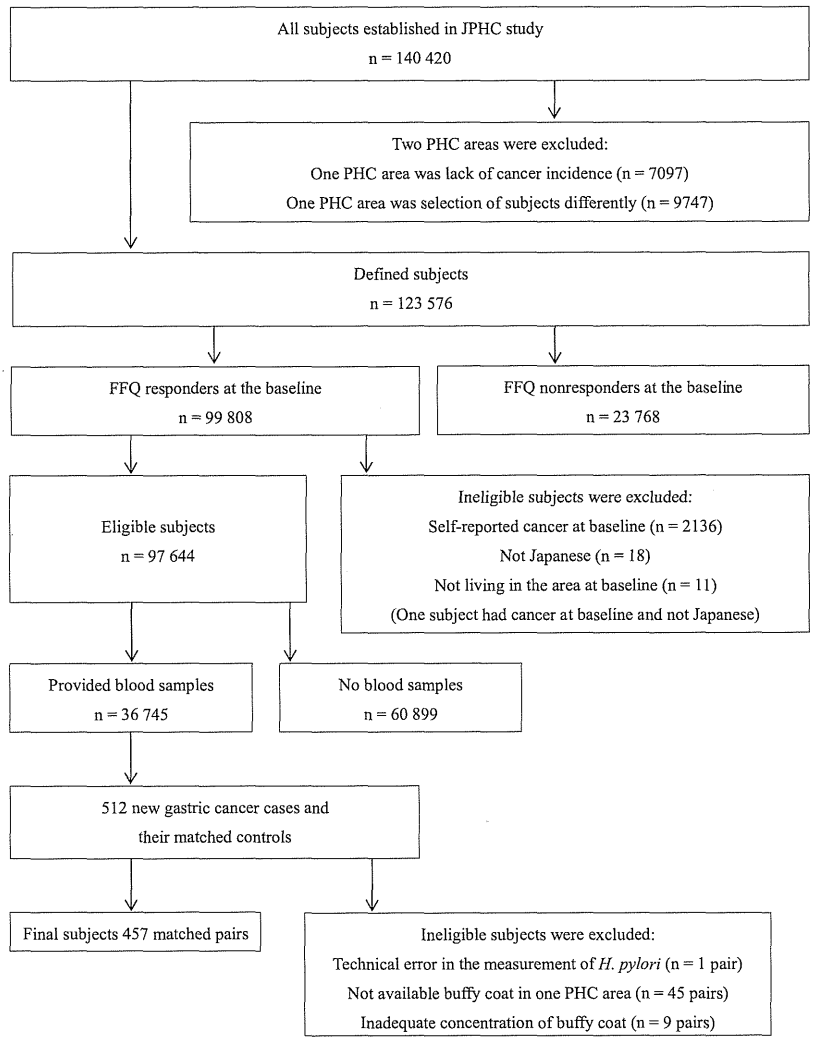

Figure 1. Flowchart of the study subjects.

Assessment of alcohol consumption

Information regarding alcohol consumption was assessed based on the frequency and amount using a validated self-administered food frequency questionnaire (FFQ). During the baseline survey, cohort I and cohort II used slightly different FFQ. In cohort I, the average frequency of alcohol consumption was reported in six categories (almost never, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week and every day). Subjects who drank at least once per week were also asked about the average amount and types of drinks. In cohort II, alcohol consumption status (never, former and current drinkers) was asked first, and then former and current drinkers were asked for more information, similar to cohort I. We then assigned a score to each category of the average frequency of consumption as follows: 1.5 for 1–2 days per week, 3.5 for 3–4 days per week, 5.5 for 5–6 days per week and 7 for every day in cohort I; and 1.5 for 1–2 days per week, 3.5 for 3–4 days per week and 6 for almost every day in cohort II. The amount of alcohol consumption was quantified in grams of ethanol by each type of beverage as follows: 180 ml of sake classified as 23 g of ethanol, 180 ml of shochu or awamori classified as 36 g, 633 ml of beer classified as 23 g, 30 ml of whiskey or brandy classified as 10 g and 60 ml wine classified as 6 g. Finally, we calculated the weekly ethanol intake, which was estimated by multiplying the quantity by the score. In our study, alcohol consumption was classified into three groups: never or occasional drinker; ethanol <150 g per week and ethanol ≥150 g per week. Alcohol consumption levels were defined by the unit *go*, the standard measure of ethanol content of alcoholic beverages in Japan. This unit equals 23 g of alcohol, the amount contained in 180 ml of sake. If a subject drinks 1 *go* every day, he or she is consuming ~150 g of ethanol per week. Validity of this FFQ-based estimated alcohol consumption was evaluated in a subsample of the JPHC study subjects who completed 28-day dietary records (DR). In cohort I, Spearman rank correlation coefficients between the FFQ and DR were 0.79 (n = 94) for men and 0.44 (n = 107) for women, respectively (13). In cohort II, these results were 0.59 (n = 176) for men and 0.40 (n = 178) for women, respectively (14).

Assessment of other potential confounding factors

Smoking status was divided into four groups: never smoker, former smoker, current smoker using ≤20 cigarettes per day and current smoker using ≥21 cigarettes per day. Body mass index (BMI) status was divided into three groups: BMI <22 kg/m^2, 22 kg/m^2 ≤ BMI <25 kg/m^2 and BMI ≥25 kg/m^2. According to a previous prospective study of the association with gastric cancer risk in Japan (15), the classifications for smoking status and BMI are reasonable. Total calorie intake and salt intake were treated as continuous variables. Family history of gastric cancer was considered positive if at least one parent or sibling had gastric cancer. The *H.pylori* infection status was regarded as positive if subjects had either *H.pylori* antibody ≥10 U/ml or cytotoxin-associated gene A (CagA) antibody >10. Atrophy was regarded

as positive if pepsinogen I was ≤70 ng/ml and pepsinogen I:pepsinogen II ratio was ≤3 (16). History of diabetes mellitus (DM) was considered positive if subjects reported a history of DM and/or drug use for DM at baseline.

Genotyping of ADH1B, ADH1C and ALDH2 polymorphisms

DNA of each subject was extracted from white blood cells in the buffy coat using a FlexiGene DNA kit (Qiagen, Hilden, Germany). Genotyping of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms was analyzed by using TaqMan single nucleotide polymorphism genotyping assays (Applied Biosystems Inc, Foster City, CA). In this assay, fluorescently labeled sequence-specific primers were used in polymerase chain reaction. These measurements were performed with blinding of case and control status. The genotype distributions of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms among controls were all in agreement with Hardy–Weinberg equilibrium ($P > 0.05$).

Statistical analysis

The chi-square test was used to compare baseline characteristics between cases and controls. Matched odds ratios (OR) and their corresponding 95% confidence intervals (CIs) were calculated to indicate the association between alcohol consumption, *ADH1B*, *ADH1C* and *ALDH2* polymorphisms, and gastric cancer risk using conditional logistic regression models. OR1 was matched for age (±3 years), sex, PHC area, blood donation date (±2 months) and fasting time at blood donation (±5 h). OR2 was further adjusted for potential confounding factors such as smoking status, alcohol consumption, total calorie intake, salt intake, BMI, family history of gastric cancer, *H.pylori* infection status, atrophy and history of DM. Data for subjects who were missing values for BMI ($n = 8$), total calorie intake ($n = 1$) and salt intake ($n = 1$) were deleted from the study when adjusting for these confounding factors. When we calculated the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption, and that of these polymorphisms combined, unconditional logistic regression models were used. We conducted the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms associated with alcohol consumption with further adjustment for these polymorphisms mutually. Reported P values were two-sided, and $P < 0.05$ was defined as statistically significant. All statistical analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC).

Results

Baseline characteristics of cases and controls are shown in Table 1. Higher BMI was more frequently distributed among controls than patients with gastric cancer. In contrast, history of DM, family history of gastric cancer, *H.pylori*, CagA positivity and atrophy were more frequently distributed among patients. These results generally agree with previous reports, including the JPHC study (15,17–19).

Table 2 presents the association between alcohol consumption, *ADH1B*, *ADH1C* and *ALDH2* polymorphisms and gastric cancer risk. Alcohol consumption was marginally associated with an increased risk of gastric cancer in the OR1 group compared with never to occasional drinkers; drinkers with ethanol <150 g/week had OR of 0.89 and with ≥150 g/week had OR of 1.29 (P for trend = 0.15). However, after further adjustment for potential confounding factors, the association became null (OR2 group). Compared with *ALDH2* GG genotype, GA and AA genotypes were marginally associated with an increased risk, with OR2 values of 1.09 (95% CI = 0.77–1.54) and 2.01 (95% CI = 0.91–4.48), respectively (P for trend = 0.18). However, *ALDH2* A allele carriers had no risk association compared with GG genotype carriers. We found no association between alcohol consumption and *ADH1B* and *ADH1C* polymorphisms. *ADH1C* GG genotype was rare in this Japanese population.

Table 3 shows the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption (gene–environmental interaction). Compared with *ADH1C* AA genotype carriers who drink 0 to <150 g/week, G allele carriers who drink ≥150 g/week had an increased risk, with OR2 value of 2.54 (95% CI = 1.05–6.17); the interaction between alcohol consumption and G allele carriers was statistically significant (P for interaction = 0.02). *ALDH2* A allele carriers who drink ≥150 g/week had an increased risk compared with GG genotype carriers who drink 0 to <150 g/week, with OR2 value of 2.08 (95% CI = 1.05–4.12). A trend toward a positive interaction between alcohol consumption and A allele carrier status was shown (P for interaction = 0.08). No association was shown for *ADH1B* polymorphism and alcohol consumption.

We further examined the effect modification of the combination of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption (gene–gene–environmental interaction) in Table 4. Compared with the combination of *ADH1B* AA and *ALDH2* GG genotype carriers who drink 0 to <150 g/week, each combination of *ADH1B* AA genotype and *ALDH2* A allele, *ADH1B* G allele and *ALDH2* A allele carriers who drink ≥150 g/week showed a trend toward an increased risk for gastric cancer, with OR2 values of 2.16 (95% CI = 0.83–5.63) and 1.66 (95% CI = 0.66–4.16), respectively. However, the interaction between *ADH1B* G allele and *ALDH2* A allele and alcohol consumption was not statistically significant (P for interaction = 0.40). In addition, compared with the combination of *ADH1C* AA and *ALDH2* GG genotype carriers who drink 0 to <150 g/week, the combination of ADH1C G and ALDH2 A alleles in carriers who drink 0 to <150 g/week showed a statistically significant decreased risk (OR = 0.43, 95% CI = 0.21–0.91). Each combination of *ADH1C* AA genotype and *ALDH2* A, *ADH1C* G and *ALDH2* A alleles in carriers who drink ≥150 g/week showed a marginally increased risk, with OR2 values 1.92 (95% CI = 0.95–3.87) and 8.95 (95% CI = 0.62–129.25), respectively. Moreover, the interaction between *ADH1C* G allele and *ALDH2* A allele and alcohol consumption seemed to be marginally statistically significant (P for interaction = 0.13).

We performed stratified analyses by sex regarding the association of each polymorphism with gastric cancer risk and observed no differences by stratification (data not shown). In addition, the gene–environmental interaction analysis was repeated with stratification by gastric atrophy. Among the subjects with gastric atrophy, *ALDH2* A allele carriers who drink ≥150 g/week had an increased risk of gastric cancer compared with those with GG genotype who drink 0 to <150 g/week (OR2 = 2.71, 95% CI = 1.18–6.27). An interaction between alcohol consumption and A allele was shown (P for interaction = 0.02). However, the subjects without gastric atrophy and *ALDH2* polymorphism did not show a positive association with risk. *ADH1B* and *ADH1C* polymorphisms also did not show any positive association with risk when stratified by atrophy. We also evaluated the combination effects of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk. Compared with *ADH1B* AA, *ADH1C* AA and *ALDH2* GG genotype carriers, OR2s were 1.15 (95% CI = 0.75–1.76) (P for interaction = 0.13) for *ADH1B* G and *ALDH2* A allele carriers and 0.59 (95% CI = 0.30–1.15) (P for interaction = 0.02) for *ADH1C* G and *ALDH2* A allele carriers. Although the interaction between *ADH1C* and *ALDH2* polymorphisms was statistically significant, a chance finding cannot be ruled out because *ADH1C* GG genotype was rare among our study subjects. Analyses considering anatomic site and histological type of gastric cancer were also performed. Cardia site ($n = 76$) was not robustly evaluated because of the small number of subjects. When limited to distal site and intestinal or diffuse type of gastric cancer, *ADH1C* G allele and *ALDH2* A allele carriers who drink ≥150 g/week showed a trend

Table 1. Baseline characteristics of cases and controls

Characteristics	Cases	Controls	P value[a]
n	457	457	
Age, mean (SD)	56.9 (7.10)	56.9 (7.12)	Matching value
Men (%)	307(67.2)	307 (67.2)	Matching value
Smoking status			
Never (%)	209 (45.7)	229 (50.1)	
Former (%)	81 (17.7)	88 (19.3)	
Current: ≤20 cigarettes/day (%)	130 (28.5)	101 (22.1)	
Current: ≥21 cigarettes/day (%)	37 (8.1)	39 (8.5)	0.18
Alcohol consumption			
Never to occasional (%)	222 (48.6)	228 (49.9)	
1+ per day and <150 g/week (%)	86 (18.8)	105 (23.0)	
1+ per day and ≥150 g/week (%)	149 (32.6)	124 (27.1)	0.12
BMI (kg/m^2)[b]			
BMI <22 (%)	168 (37.1)	141 (31.1)	
22≤ BMI <25 (%)	193 (42.6)	191 (42.2)	
25≤ BMI (%)	92 (20.3)	121 (26.8)	0.04
History of DM (%)	41 (9.0)	19 (4.2)	0.005
Family history of gastric cancer (%)	53 (11.6)	31 (6.8)	0.02
Helicobacter pylori-positive (%)[c]	428 (93.7)	341 (74.6)	<0.001
CagA-positive (%)	349 (76.4)	318 (69.6)	0.03
Atrophy (%)[d]	375 (82.1)	261 (57.1)	<0.001

[a]Based on chi-square test.
[b]Subject data without calculated BMI data because of missing values for height or weight in four cases and four controls were deleted.
[c]Based on immunoglobulin G antibody.
[d]Atrophy: positive if pepsinogen I ≤70 ng/ml and pepsinogen I:pepsinogen II ratio ≤3.

Table 2. Association between alcohol consumption, *ADH1B*, *ADH1C* and *ALDH2* polymorphisms, and gastric cancer risk

	Genotype frequency (%)[a]	Cases (*n*)/controls (*n*)	OR1 (95% CI)[b]	OR2 (95% CI)[c]
Alcohol consumption[d]				
Never to occasional		222/228	1.00 (reference)	1.00 (reference)
1+ per day and <150 g/week		86/105	0.89 (0.60–1.33)	0.73 (0.46–1.17)
1+ per day and ≥150 g/week		149/124	1.29 (0.88–1.89)	1.09 (0.68–1.74)
P for trend			0.15	0.64
ADH1B (rs1229984)				
AA	55.6	252/254	1.00 (reference)	1.00 (reference)
AG	36.8	173/168	1.03 (0.78–1.36)	0.93 (0.67–1.29)
GG	7.6	32/35	0.92 (0.56–1.51)	0.88 (0.50–1.54)
P for trend			0.92	0.56
AG+GG	44.4	205/203	1.01 (0.78–1.31)	0.91 (0.67–1.24)
ADH1C (rs698)				
AA	85.6	396/391	1.00 (reference)	1.00 (reference)
AG	14.2	60/65	0.91 (0.63–1.33)	0.79 (0.51–1.21)
GG	0.2	1/1	1.00 (0.06–15.99)	1.51 (0.02–97.99)
P for trend			0.65	0.26
AG+GG	14.4	61/66	0.90 (0.62–1.30)	0.79 (0.51–1.22)
ALDH2 (rs671)				
GG	63.9	287/292	1.00 (reference)	1.00 (reference)
GA	32.8	149/150	0.99 (0.74–1.32)	1.09 (0.77–1.54)
AA	3.3	21/15	1.33 (0.67–2.61)	2.01 (0.91–4.48)
P for trend			0.68	0.18
GA+AA	36.1	170/165	1.02 (0.77–1.34)	1.16 (0.83–1.62)

Based on conditional logistic regression model.
[a]Among controls.
[b]Matched for age (±3 years), sex, area, blood donation date (±2 months) and fasting time at blood donation (±5 h).
[c]Further adjusted for smoking status, alcohol consumption, body mass index, total calorie, salt intake, family history of gastric cancer, *Helicobacter pylori* infection status, atrophy and history of DM.
[d]Not adjusted for alcohol consumption.

toward having an increased risk relative to those who drink 0 to <150 g/week (data not shown). When we evaluated heavy drinkers who drink ≥300 or ≥450 g/week, similar associations were observed (data not shown).

Discussion

In our population-based, nested, case–control study, we observed no association between alcohol consumption, *ADH1B*

Table 3. Effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption

	ADH1B (rs1229984)						
	AA			AG+GG			
	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	P for interaction[b]
Alcohol consumption							
0 to <150 g/week	167/189	1.00 (reference)	1.00 (reference)	141/144	1.09 (0.79–1.49)	1.01 (0.72–1.42)	0.76
≥150 g /week	85/65	1.57 (1.04–2.38)	1.37 (0.86–2.19)	64/59	1.30 (0.84–2.02)	1.25 (0.77–2.05)	
	ADH1C (rs698)						
	AA			AG+GG			
	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	P for interaction[b]
Alcohol consumption							
0 to <150 g/week	270/277	1.00 (reference)	1.00 (reference)	38/56	0.68 (0.43–1.07)	0.66 (0.41–1.08)	0.02
≥150 g/week	126/114	1.23 (0.87–1.72)	1.16 (0.78–1.71)	23/10	2.49 (1.14–5.42)	2.54 (1.05–6.17)	
	ALDH2 (rs671)						
	GG			GA+AA			
	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[c]	Cases (*n*)/controls (*n*)	OR1 (95% CI)[a]	OR2 (95% CI)[c]	P for interaction[c]
Alcohol consumption							
0 to <150 g/week	177/185	1.00 (reference)	1.00 (reference)	131/148	0.93 (0.68–1.28)	0.98 (0.69–1.38)	0.08
≥150 g/week	110/107	1.16 (0.79–1.69)	1.09 (0.72–1.67)	39/17	2.51 (1.34–4.72)	2.08 (1.05–4.12)	

Based on unconditional logistic regression model.

[a]Adjusted for age (±3 years), sex, area, blood donation date (±2 months) and fasting time at blood donation (±5 h).

[b]Further adjusted for smoking status, BMI, total calorie, salt intake, family history of gastric cancer, *H.pylori* infection status, atrophy, history of DM and *ALDH2* polymorphism.

[c]Further adjusted for smoking status, BMI, total calorie, salt intake, family history of gastric cancer, *H.pylori* infection status, atrophy, history of DM and *ADH1B* and *ADH1C* polymorphisms.

Table 4. Effect modification of the combination of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption

		ALDH2 (rs671)						
		GG			GA+AA			
		Cases (n)/controls (n)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	Cases (n)/controls (n)	OR1 (95% CI)[a]	OR2 (95% CI)[b]	P for interaction[b]
ADH1B (rs1229984)	Alcohol consumption							
AA	0 to <150 g/week	103/105	1.00 (reference)	1.00 (reference)	64/84	0.77 (0.50–1.19)	0.75 (0.47–1.19)	0.40
	≥150 g/week	64/58	1.20 (0.74–1.94)	0.99 (0.58–1.68)	21/7	3.09 (1.23–7.76)	2.16 (0.83–5.63)	
AG+GG	0 to <150 g/week	74/80	0.91 (0.60–1.39)	0.79 (0.50–1.24)	67/64	1.07 (0.68–1.66)	1.06 (0.65–1.71)	
	≥150 g/week	46/49	1.01 (0.60–1.69)	0.94 (0.53–1.65)	18/10	1.93 (0.83–4.46)	1.66 (0.66–4.16)	
ADH1C (rs698)	Alcohol consumption							
AA	0 to <150 g/week	152/161	1.00 (reference)	1.00 (reference)	118/116	1.07 (0.76–1.51)	1.13 (0.78–1.63)	0.13
	≥150 g/week	92/98	1.07 (0.72–1.59)	1.01 (0.65–1.57)	34/16	2.34 (1.21–4.51)	1.92 (0.95–3.87)	
AG+GG	0 to <150 g/week	25/24	1.03 (0.56–1.90)	1.03 (0.54–1.99)	13/32	0.44 (0.22–0.87)	0.43 (0.21–0.91)	
	≥150 g/week	18/9	2.20 (0.94–5.14)	2.14 (0.83–5.52)	5/1	5.63 (0.64–49.22)	8.95 (0.62–129.25)	

Based on unconditional logistic regression model.

[a]Adjusted for age (±3 years), sex, area, blood donation date (±2 months) and fasting time at blood donation (±1 h).

[b]Further adjusted for smoking status, BMI, total calorie, salt intake, family history of gastric cancer, *H.pylori* infection status, atrophy and history of DM.

(rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms and gastric cancer risk. However, statistically significant interactions between inactive *ADH1C* and alcohol consumption and nonsignificant interactions between inactive *ALDH2* alleles and alcohol consumption were shown for gastric cancer risk.

To date, one prospective study in Europe (20) and several case–control studies (21–25) have reported an association between alcohol consumption and *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms and gastric cancer risk. For *ADH1B* (rs1229984) and *ADH1C* (rs698) polymorphisms, two previous studies reported that the inactive *ADH1B* allele was not associated with gastric cancer risk among drinkers (20,23). In one case–control study performed in the United States, the active *ADH1C* genotype was associated with an increased risk among drinkers and nondrinkers (24). However, the sample size of this case–control study was small, and caution may be needed in interpreting the results. For the *ALDH2* (rs671) polymorphism, two recent, large, Japanese and Korean case–control studies reported that the interaction between the inactive *ALDH2* allele and alcohol consumption regarding gastric cancer risk was statistically significant (21,22). Another case–control study conducted in China also indicated that inactive *ALDH2* allele carriers with larger cumulative amount of alcohol consumption had a marginally increased risk of gastric cancer compared with active *ALDH2* allele carries with smaller cumulative amount of alcohol consumption (23).

As shown in our study (Tables 3 and 4), the association between alcohol consumption, *ADH1B* polymorphism and gastric cancer risk was similar to that in previous studies. In contrast, a positive association between inactive *ADH1C* G allele and alcohol consumption regarding gastric cancer risk was found, opposite to that found in previous studies (24). However, the number of study subjects in our population is small for some *ADH1C* genotypes because *ADH1C* GG genotype is rare in Asians (8). In addition, evidence is lacking on any difference between *ADH1B* and *ADH1C* polymorphisms in the ability to metabolize ethanol. Caution is necessary when interpreting the results for *ADH1C* polymorphisms in our study. Inactive *ALDH2* A allele carriers who drink ≥150 g/week have an increased risk of gastric cancer, similar to that in previous studies, which may be attributable to accumulation of acetaldehyde. When subjects with an inactive *ALDH2* allele did not drink alcohol, the risk of gastric cancer did not increase. We suggest that accumulation of acetaldehyde modified by *ALDH2* (rs671), rather than *ADH1B* (rs1229984) and *ADH1C* (rs698) polymorphisms, may play an important role in gastric carcinogenesis.

It has been reported that the carcinogenic mechanisms of acetaldehyde are complicated and are not completely understood. Acetaldehyde reacts with the exocyclic amino group of deoxyguanosine to form DNA adducts, called N (2)-ethylidenedeoxyguanosine [N (2)- ethylidene-dGuo]. The DNA adducts are involved in metagenesis (26,27). The other kinds of acetaldehyde-related adducts are the 1,N (2)-propano-2′-deoxyguanosine [1, N (2)-propanodGuo] and 1,N (2)-etheno-dGuo (27). Other candidate mechanisms may be DNA hypomethylation by DNA methyltransferase, direct adduction of histone, and inhibition of the activity of O6-methylguanine-DNA methyltranseferase (MGMT), which protect against alkylation of DNA (27).

In our study, we conducted a stratified analysis of gastric atrophy. Interestingly, among the subjects with gastric atrophy, *ALDH2* A allele carriers who drink ≥150 g/week had an increased risk of gastric cancer compared with GG genotype carriers who drink 0 to <150 g/week; statistically significant interaction was also found. This phenomenon was not found among the

subjects without gastric atrophy. Our result suggests that acetaldehyde may induce gastric carcinogenesis with gastric atrophy, which is caused by chronic inflammation with *H.pylori* infection. In a previous study, although statistical interaction was not significant, similar results were found (21). Further studies are needed to clarify the contribution of acetaldehyde to gastric carcinogenesis.

This study has several strengths. First, this is a population-based prospective study, which is more reliable than case–control studies. Detailed information regarding the potential confounding factors including alcohol consumption was recorded before diagnosis of gastric cancer, thus confirming our results. A validated FFQ was used. Also, we were able to control potential confounding factors, as compared with a previous European study that was only adjusted for age, sex and country (20).

Our study does have some weakness. First, among 97 644 eligible subjects of the JPHC study, only 36 745 (37.6%) subjects provided blood samples. The participants in the health check-up survey relative to nonparticipants had a favorable lifestyle with less smoking and alcohol consumption, as reported previously (28). Second, we were not able to assess the other genes of ethanol-metabolizing enzymes such as *CYP2E1*. Third, we analyzed the gastric cancer risk only using the lifestyle information at baseline. Lifestyle habits of study subjects might change during the follow-up period. However, this change may not be different between cases and controls and likely would have led to the underestimation of results. Finally, sample size was not necessarily enough for evaluating the association among some anatomic sites.

In conclusion, to find the relation between alcohol consumption and gastric cancer risk, it is important to consider both alcohol consumption level and *ADH1C* and *ALDH2* polymorphisms. However, caution is needed to interpret the results associated with the *ADH1C* polymorphism because some genotypes of the *ADH1C* polymorphism occurred in only a small number of subjects.

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Appendix

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特集 大腸癌のバイオマーカー

Gastrointestinal Research

血清メタボローム解析にもとづく早期大腸癌診断のバイオマーカー

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Summary
大腸癌は癌による死亡者数の上位を占めている．また，検査方法として便潜血検査や大腸内視鏡検査がおこなわれているが，その診断能や侵襲度の高さが問題となっており，より簡便で低侵襲な診断法の確立が求められている．近年，オミックス解析の一つとしてメタボロミクスが注目されている．メタボロームは，その特徴として生体の現在の病態をリアルタイムに反映することなどから，疾患のバイオマーカーの探索に適していると考えられる．今回，われわれはガスクロマトグラフィー質量分析（GC/MS）を使用したメタボロミクスを用いて4種の代謝物から構成される大腸癌の診断モデルを作成した．この診断モデルは感度，特異度ともに85%と高く，一般的に使用されている腫瘍マーカー（CEA，CA19-9）と比較しても高い診断能を有しており，とくに早期大腸癌（Stage Ⅰ～Ⅱ）の症例でも高い感度を示した．今後，より詳細，かつ，大規模な研究が進むことで，メタボロミクスが臨床的に高い診断能を有する検査として期待される．

Key words
メタボロミクス バイオマーカー 質量分析（MS） 大腸癌

はじめに

大腸癌は2012年の時点で，わが国における男性の死亡者数第3位，女性の死亡者数第1位と非常に高い死亡者数を示しており今後も増加傾向が予想され，国民病となりつつある[1]．また，癌を早期の状態で発見することができれば，低侵襲な治療で根治が期待でき，治療後の患者のQOLの大きな改善が望まれる．スクリーニング検査での罹患者の拾い上げは非常に重要であり，大腸癌の早期発見を目的とするスクリーニング法（一次検査）として便潜血検査が広く普及している．便潜血検査は簡便，安価であるというメリットがあるが，その一方，デメリットとして感度は免疫法便潜血検査で，1日法で56～66%，2日法で77～88%とスクリーニング法としては感度が低く，痔などの疾患による偽陽性もしばしば問題となる[2]．また，精密検査（二次検査）としては大腸内視鏡検査がおこなわれている．大腸内視鏡検査は感度が高く，生検による組織診断やポリープの切除など

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