JDS/JCA Joint Recommendations on Diabetes and Cancer for the General Public (Including Patients)

- Diabetes (mainly type 2 diabetes) in Japanese is associated with an increased risk of colorectal, liver, and pancreatic cancers. There is as yet no consensus as to whether or not diabetes is associated with an increased risk of other types of cancer.
- Healthy diet, exercise, body weight control, smoking cessation, and alcohol moderation may help prevent cancer and are therefore encouraged.
- Diet/exercise therapy, smoking cessation, and alcohol moderation may help prevent cancer in patients with diabetes as well.
- Patients with diabetes are encouraged to undergo evidence-based screening as required, depending on their sex and age (Table 2). They are also encouraged to visit a hospital to undergo screening for liver cancer if they are hepatitis virus-positive.
- At present, there is no clear consensus as to whether or not a particular antidiabetic drug is associated with cancer risk. It is therefore essential that diabetic patients focus attention on maintaining favorable glycemic control following their physicians' instructions.

Disclosure Statement

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Impact of five modifiable lifestyle habits on the probability of cancer occurrence in a Japanese population-based cohort: Results from the JPHC study

Hadrien Charvat [a], Shizuka Sasazuki [a,*], Manami Inoue [a,b], Motoki Iwasaki [a], Norie Sawada [a], Taichi Shimazu [a], Taiki Yamaji [a], Shoichiro Tsugane [a], for the JPHC Study Group

[a] Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan
[b] Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

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ABSTRACT

Objective. The present work aims to provide 10-year estimates of the probability of cancer occurrence in the Japanese population based on age, sex, and the pattern of adherence to five healthy lifestyle habits.

Methods. The study population consisted of 74,935 participants in the Japan Public Health Center-Based Prospective Study (aged 45 to 74 years) who answered a 5-year follow-up questionnaire about various lifestyle habits between 1995 and 1999. The relationship between five previously identified healthy lifestyle habits (never smoking, moderate or no alcohol consumption, adequate physical activity, moderate salt intake, and appropriate body mass index) and cancer occurrence was assessed using a sex-specific parametric survival model.

Results. Compared to individuals not adhering to any of the five habits, never-smoking men had a nearly 30% reduction in the 10-year probability of cancer occurrence (e.g., 20.5% vs. 28.7% at age 70), and never-smoking women had a 16% reduction (e.g., 10.5% vs. 12.5% at age 70). Adherence to all five habits was estimated to reduce the 10-year probability of cancer occurrence by 1/2 in men and 1/3 in women.

Conclusion. By quantifying the impact of lifestyle habits on the probability of cancer occurrence, this study emphasizes the importance of lifestyle improvement.

Introduction

Lifestyle habits such as cigarette smoking and alcohol consumption play an important role in the development of several cancers as well as other health conditions such as cardiovascular disease. Because these habits are modifiable, they constitute particularly attractive targets for public health policies.

We previously estimated the effect of adherence (or lack thereof) to five identified healthy lifestyle habits—never smoking, moderate or no alcohol consumption, moderate salt intake, adequate physical activity, and appropriate body mass index (BMI) (Inoue et al., 2004a,b, 2005, 2008a; Takachi et al., 2010)—on the risk of the occurrence of cancer in the Japanese population (Sasazuki et al., 2012). The findings were expressed as hazard ratios and population attributable fractions, two indicators that are commonly used to report results from association studies. Hazard ratios provide insight into the relationship between exposure to a risk factor and the probability of occurrence of a particular health outcome. In addition, attributable fractions (Eide and Heuch, 2001; Walter, 1976) summarize the impact of a causal risk factor in terms of the proportion of cases of the disease that could theoretically be prevented in the population by suppressing or reducing exposure to that risk factor. However, neither indicator conveys how making lifestyle improvements would benefit individuals in terms of the impact on their own health.

The need for better tools for reporting results from basic studies and for emphasizing their practical implications has recently been stressed (Suenaga et al., 2012). As part of our translational research, the present work extends our previous analysis (Sasazuki et al., 2012) of the beneficial effect of five modifiable healthy lifestyle habits by providing estimates of the probability of developing cancer based on the pattern of adherence to these habits.

Methods

Study population

Details of the study design have been described elsewhere (Tsugane and Sobue, 2001). Briefly, the participants in the present study were Japanese individuals included in the Japan Public Health Center (JPHC)-Based Prospective Study who answered a 5-year follow-up questionnaire about lifestyle habits during the period from 1995 to 1999 and who were subsequently followed until 31 December 2006. The institutional review board of the National Cancer

* Corresponding author at: Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Fax: +81 3 3547 8578.
E-mail address: ssasazuk@ncc.go.jp (S. Sasazuki).

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Table 1
Number of cases of the five most frequent cancers identified for each gender among 74,935 individuals from the Japan Public Health Center-Based Prospective Study (1995).

Men (n = 3213)		Women (n = 2046)	
Cancer sites (ICD-O-3 codes)	Number (%)	Cancer sites (ICD-O-3 codes)	Number (%)
Stomach (C16)	698 (21.7%)	Colorectal (C18, C19, C20)	401 (19.6%)
Colorectal (C18, C19, C20)	603 (18.8%)	Breast (C50)	349 (17.1%)
Lung (C34)	461 (14.3%)	Stomach (C16)	287 (14.0%)
Prostate (C61)	385 (12.0%)	Lung (C34)	197 (9.6%)
Liver (C22)	206 (6.4%)	Liver (C22)	90 (4.4%)

Center, Tokyo, Japan, approved the study. Individuals from the Katsushika area of Tokyo were excluded.

Follow-up and identification of cancer cases

In Japan, residency and death registrations are required by the Basic Residential Register Law and the Family Registry Law, respectively, and registries are thought to be exhaustive. Incident cancer cases were identified by active patient notification from major local hospitals in each study area and from data linkage with population-based cancer registries. Cancer cases were coded according to the *International Classification of Diseases for Oncology*, 3rd edition (World Health Organization, 2000). In our cancer registry system, the proportion of cases for which information was available only through death certificates was 6.1%.

Dataset

The original population consisted of 133,323 individuals recruited during the period from 1990 to 1994. For the present study, the starting point was defined as the date of questionnaire completion or the date of entry plus 5 years for individuals who did not answer the questionnaire. Individuals with non-Japanese nationality (n = 51), incorrect birth date (n = 7), or duplicate enrolment (n = 4) or who emigrated before the starting point (n = 185) were excluded. Removal of 12,067 individuals who had died (n = 3430), moved away from the study area (n = 8245), or been lost to follow-up before the starting point (n = 392) yielded a population of 121,009 eligible individuals. Among these, 98,456 answered the questionnaire (response rate of 81.4%). Individuals diagnosed with or reported as having cancer (n = 2156), cardiovascular disease (n = 1188), or ischaemic heart disease (n = 1471) before the starting point; who had missing data regarding smoking (n = 4702), alcohol consumption (n = 2439), or physical activity (n = 4490); who had missing or inadequate data for BMI (n = 2822); or who reported extreme total energy intake (2.5th and 97.5th percentile for each sex; n = 5820) were also excluded. Because diabetes mellitus is related to most of the lifestyle habits identified and may also be related to the occurrence of certain types of cancer, we excluded individuals with a reported personal history of diabetes (n = 3613). The final dataset included 74,935 individuals (34,635 men and 40,300 women) aged 45 to 74 years.

Healthy lifestyle habit definition

The healthy lifestyle habits were defined as follows: individuals were considered never smokers if they had never smoked; moderate or no alcohol consumption was defined as a weekly alcohol consumption of <150 g (includes non-drinkers); moderate salt intake was defined as consumption of <0.67 g of fish roe per day; adequate physical activity was defined as ≥37.5 and ≥31.9 metabolic equivalent hours per day for men and women, respectively; and appropriate BMI was defined as a BMI within the range 21–27 for men and 19–25 for women. A more detailed description of how these lifestyle habits were assessed in the questionnaire, as well as the rationale underlying the specific cut-off points, can be found in Sasazuki et al. (2012). Table 1 summarizes the sex-specific distribution of the number of lifestyle habits adhered to.

Statistical analyses

The occurrence of cancer over time was modeled with a sex-specific flexible parametric survival model (Cluze et al., 2009; Remontet et al., 2007). Person-years of follow-up for each individual were calculated from the starting point to the date of cancer diagnosis, date of emigration from the study area, date of death, or end of follow-up (31 December 2006), whichever came first. The logarithm of the baseline hazard was modeled as a parametric function of time. Age at the start of the study was introduced into the model as a continuous variable, and its effect was assessed by means of different parametric functions. Moreover, the non-proportional effect of age was assessed and, if necessary, modeled through the inclusion of terms for the interaction between age and the logarithm of the baseline hazard. The five lifestyle habits were included in the model, and interactions between habits, and between habits and age were tested. Because the consumption of salt-preserved food is highly correlated with area of residence (individuals in northern Japan tend to have a higher salt intake than those in southern Japan), we did not adjust the model for geographical area, as had been done previously (Sasazuki et al., 2012). The selection of the appropriate parametric function (linear, quadratic, or quadratic spline with one knot) for the baseline hazard and the effect of age, and the inclusion of a potential non-proportional effect of age, were based on likelihood ratio tests. Using the obtained model, we estimated the probability of cancer occurrence at 10 years according to sex, age, and pattern of adherence to the lifestyle habits. Ninety-five per cent confidence intervals were calculated using the delta method.

The predictive performance of the model was assessed in terms of discrimination and calibration (Moons et al., 2012). Discrimination was estimated using the generalization of Harrell's c-index (Harrell et al., 1996; Pencina and D'Agostino, 2004) to non-proportional hazard models described by Antolini et al. (2005) using 10 years as the reference follow-up time. Internal validation was provided by means of bootstrapping. For each dataset (men or women), 500 bootstrap samples were generated, and for each of these, the parameters of the previously obtained sex-specific model were re-estimated. With these parameters, the c-index was calculated for the bootstrap sample and estimated for the original dataset. The optimism was then estimated as the average of the difference between the two c-index values. Finally, the optimism-corrected c-index was obtained by subtracting the estimated optimism from the apparent c-index (i.e., that obtained with the original model on the original dataset) (Harrell et al., 1996). Calibration was assessed through the analog of the Hosmer–Lemeshow's chi-square test for survival analysis developed by D'Agostino and Nam (2004) using 10 years as the endpoint and dividing the study population into deciles of predicted risk. All analyses were carried out with R statistical software (ver. 2.15.0, R Development Core Team, 2012).

Results

A total of 3213 cases of cancer occurred in men during 315,528.4 person-years of follow-up, and 2046 cases occurred in women during 380,147.1 person-years. Table 1 shows the number of cancer cases for the five most frequent localizations in men and women that

Table 2
Distribution of the number of healthy lifestyle habits adhered to according to sex among 74,935 individuals from the Japan Public Health Center-Based Prospective Study (1995).

Number of healthy lifestyle habits	Men (n = 34,635)		Women (n = 40,300)	
	Number (%)	Number of cancer cases (proportion in %)	Number (%)	Number of cancer cases (proportion in %)
0	730 (2.11)	97 (13.29)	25 (0.06)	1 (4.00)
1	4671 (13.49)	523 (11.20)	245 (0.61)	16 (6.53)
2	10,859 (31.35)	1068 (9.84)	2452 (6.08)	152 (6.20)
3	11,525 (33.28)	1000 (8.68)	11,503 (28.54)	631 (5.49)
4	5864 (16.93)	450 (7.67)	17,926 (44.48)	883 (4.93)
5	986 (2.85)	75 (7.61)	8149 (20.22)	363 (4.45)

Table 3
Hazard ratios associated with adherence to the five healthy lifestyle habits, determined by means of a parametric multivariable survival model with nonlinear and non-proportional effects of age for men and women.

Lifestyle habit	Men		Women	
	HR[a]	95% CI[b]	HR	95% CI
Never smoking	0.68	[0.62–0.73]	0.82	[0.69–0.98]
Moderate or no drinking	0.87	[0.81–0.93]	0.94	[0.72–1.24]
Adequate physical activity	0.89	[0.82–0.97]	0.88	[0.81–0.96]
Moderate salt intake	0.90	[0.83–0.98]	0.95	[0.85–1.05]
Appropriate BMI	0.97	[0.90–1.04]	0.93	[0.85–1.01]

[a] HR: hazard ratio.
[b] CI: confidence interval.

account for 73.2% and 64.7%, respectively, of all cancer cases diagnosed in the cohort. Except for prostate and breast cancer, the most frequent cancers are the same for men and women, albeit in a different order: stomach, colorectal, lung and liver cancer.

Table 2 shows the distribution of individuals according to the number of healthy lifestyle habits adhered to, along with the corresponding number of cancer cases. The stratum-specific prevalence of cancer tended to decrease with the number of healthy lifestyle habits, particularly in men.

Table 3 presents the effects, measured in terms of hazard ratios, of the healthy lifestyle habits on the development of cancer after the time-dependent effect of age was taken into account. The most influential habits in terms of prevention of cancer occurrence were never smoking, moderate or no drinking, and adequate physical activity for men and never smoking and adequate physical activity for women. A non-significant reduction of risk was associated with appropriate BMI in men and with moderate or no drinking, moderate salt intake, and appropriate BMI for women. These results are similar to those described in Sasazuki et al. (2012).

Tables 4 and 5 show the 10-year probabilities of developing cancer for men and women, respectively, according to age and adherence to each of the five lifestyle habits. Moreover, the tables also show the probability estimates obtained by combining healthy lifestyle habits sequentially from the most to the least effective in terms of risk reduction. Note that the sequences differed for men and women. Probability estimates for all possible combinations of the five healthy habits are given in Appendix B.

Comparison of the data presented in Tables 4 and 5 for individuals not adhering to any of the healthy lifestyle habits reveals that, except at the younger ages, the 10-year probability of cancer occurrence in men was almost twice that in women at the same age (e.g., 16.45% vs. 8.31% at age 60, and 29.48% vs. 15.46% at age 75). Moreover, the 10-year probability of developing cancer increased more steeply with age in men between 50 and 70 (5.82% at age 50 vs. 28.68% at age 70) than in women between the same ages (5.47% at age 50 vs. 12.58% at age 70).

Table 4
Ten-year probability (%) of developing cancer for men according to age and pattern of adherence to healthy lifestyle habits.

Pattern of adherence	Age (years)						
	45	50	55	60	65	70	75
None	3.87	5.82	9.62	16.45	23.73	28.68	29.48
Never smoking (1)	2.64	3.98	6.62	11.46	16.75	20.45	21.06
Moderate or no drinking (2)	3.37	5.07	8.40	14.44	20.94	25.41	26.14
Adequate physical activity (3)	3.47	5.22	8.65	14.84	21.50	26.07	26.81
Moderate salt intake (4)	3.50	5.27	8.73	14.99	21.70	26.31	27.06
Appropriate BMI (5)	3.74	5.63	9.31	15.94	23.02	27.85	28.63
(1) + (2)	2.29	3.46	5.77	10.02	14.71	18.00	18.54
(1) + (2) + (3)	2.05	3.10	5.17	9.00	13.25	16.25	16.75
(1) + (2) + (3) + (4)	1.85	2.80	4.68	8.17	12.05	14.80	15.26
(1) + (2) + (3) + (4) + (5)	1.79	2.71	4.53	7.90	11.66	14.33	14.78

Table 5
Ten-year probability (%) of developing cancer for women according to age and pattern of adherence to healthy lifestyle habits.

Pattern of adherence	Age (years)						
	45	50	55	60	65	70	75
None	4.45	5.47	6.74	8.31	10.23	12.58	15.46
Never smoking (1)	3.67	4.52	5.57	6.88	8.48	10.46	12.89
Adequate physical activity (2)	3.93	4.84	5.96	7.35	9.07	11.17	13.75
Appropriate BMI (3)	4.12	5.07	6.25	7.71	9.50	11.70	14.39
Moderate or no drinking (4)	4.21	5.18	6.38	7.87	9.69	11.93	14.67
Moderate salt intake (5)	4.21	5.19	6.40	7.88	9.71	11.96	14.70
(1) + (2)	3.24	3.99	4.93	6.08	7.51	9.28	11.44
(1) + (2) + (3)	3.00	3.70	4.57	5.64	6.97	8.61	10.64
(1) + (2) + (3) + (4)	2.84	3.50	4.32	5.34	6.60	8.16	10.08
(1) + (2) + (3) + (4) + (5)	2.69	3.32	4.10	5.06	6.26	7.74	9.57

Although information on the effects of the five healthy lifestyle habits on the risk of cancer is reflected in the above-mentioned hazard ratios, these effects can also be described in terms of differences in the 10-year probabilities of developing cancer. Tables 4 and 5 reveal, for example, that never smoking was associated with a nearly 30% reduction of that probability in men (e.g., 20.45% vs. 28.68% at age 70), whereas the reduction was only 16% in women (e.g., 10.46% vs. 12.58% at age 70). Adherence to all five healthy lifestyle habits nearly halved the 10-year probability of developing cancer in men (e.g., 7.90% vs. 16.45% at age 60, and 14.78% vs. 29.48% at age 75), whereas it reduced the corresponding probabilities in women by about 40% (e.g., 5.06% vs. 8.31% at age 60, and 9.57% vs. 15.46% at age 75).

In terms of predictive performance, the models showed a generalized c-index at 10 years of 0.6922 (optimism-corrected value: 0.6917) for men and 0.5942 (optimism-corrected value: 0.5920) for women. In terms of calibration, the D'Agostino and Nam chi-square test was non-significant for both models ($\chi^2 = 10.96$, $p = 0.278$ for men; $\chi^2 = 11.95$, $p = 0.216$ for women), suggesting good agreement between observed and predicted probabilities of cancer occurrence by deciles of predicted risk.

Discussion

The development of chronic diseases such as cancer is seldom the result of a single cause; instead, a complex combination of genetic susceptibilities and exposures to various risk factors during the course of a person's life is thought to be responsible for cell transformation and tumor development. In particular, risk factors related to lifestyle have recently come to prominence because they are widespread and amenable to modification (Haveman-Nies et al., 2002; Ma et al., 2010; Sasazuki et al., 2012; Tamakoshi et al., 2009; Tanaka et al., 2009). The present work provides a means of quantifying the protective effects associated with five previously identified healthy lifestyle habits (Inoue et al., 2004a,b, 2005, 2008a; Takachi et al., 2010) in terms of reducing the probability of developing cancer over a given period of time.

Primary prevention of chronic diseases relies on long-term modifications of lifestyle habits; these modifications are effective at the population level but offer limited immediate benefit at the individual level. This is what Rose called the prevention paradox (Rose, 1985) and partly accounts for the difficulty in motivating individuals to change their lifestyles. Therefore, efforts should be directed toward better ways to communicate the results of epidemiological studies to the general public (Spiegelhalter et al., 2011; Suenaga et al., 2012). For this purpose, 10-year probabilities of cancer occurrence allow individuals to gauge the potential health impacts of their lifestyle choices in a more tangible way than relative risks do. The use of such probabilities along with other measures of risk (Timmermans et al., 2008) in the context of web-based applications may help to reinforce the benefit of preventive measures (Kim et al., 2004).

One strength of this work relates to the modeling procedure. The use of a flexible parametric survival model offers the advantage of providing estimates of the probability of cancer development at any point in time without the traditional reliance on Kaplan–Meier estimates (D'Agostino et al., 2001). Moreover, it allows the modeling of time-dependent effects of covariates (Abrahamowicz et al., 1996), which might be especially useful for modeling the effect of age (Cluze et al., 2009). With the continuing improvement of computational power, there should be no impediment to the more generalized use of such models (Remontet et al., 2007; Royston et al., 1999). Further methodological developments could be considered, such as using more realistic definitions of exposures, taking cancer site-specific effects of lifestyle habits into account (Ma et al., 2010), or considering competing risks between different health outcomes (Tanaka et al., 2009).

Note that we considered cancer as a global disease in this study. Several reasons explain this choice. First of all, cancer is a group of diseases characterized by common biological mechanisms including alterations of DNA and metabolic modifications leading to an uncontrolled cellular growth (Hanahan and Weinberg, 2011). Lifestyle habits can affect these mechanisms in various ways: direct mutagenic effects, modification of the metabolism of other carcinogens, modulation of the hormonal balance (Calle and Kaaks, 2004; Hecht, 2003; Seitz and Stickel, 2007; World Cancer Research Fund/American Institute for Cancer Research, 2007) so that it is legitimate to study the relationship between lifestyle habits and 'cancer' as a whole. This also makes sense from a public health point of view because people usually perceive cancer as a global disease, and focusing on particular localizations might alter the impact of the prevention message. Finally, the estimation of cancer site-specific probabilities requires careful adjustments for different sets of risk factors (e.g., hepatitis infection for liver cancer and *Helicobacter pylori* infection for gastric cancer), which was beyond the scope of the present study.

Limitations of the study

Though the modeling approach may prove successful in describing the baseline hazard as well as the effect of age on the probability of cancer occurrence, the assessment of the effect of lifestyle habits may suffer from the way they were defined, which relied on dichotomization. Altman and Royston (2006) have advocated against dichotomizing continuous variables on the grounds that it may result in a serious loss of information, may increase the risk of false-positive results, and may conceal any non-linear effects of covariates. In addition, the distribution of exposure to the five identified healthy lifestyle habits in the cohort population (Sasazuki et al., 2012) showed that more than 90% of women were never smokers and non- or moderate drinkers according to our definitions, and this fact may explain the relatively low impact of these two well-described cancer risk factors on the probability of cancer occurrence in women and the low c-index value of the model developed for women. Moreover, Table 1 shows that nearly 50% of the men adhered to two or fewer healthy habits, whereas more than 90% of the women adhered to three or more healthy habits. This fact suggests that even if the model developed for women is appropriate, it may be of limited use because most women already have healthy lifestyles according to the definition of the exposure categories. Even though the developed model shows satisfactory results in terms of predictive performance, the absence of external validation may hamper the generalizability of our findings beyond the study population. Furthermore, we must stress that the estimated differences in probabilities of cancer development apply to individuals who are considered to have consistently adhered to a particular lifestyle habit. Although the results of the present study may provide an incentive to improve one's lifestyle, they do not guarantee that modifying bad habits will reduce risk to the extent predicted, because the model fails to account for the cumulative effect of past exposures.

Because the analysis was limited to the identified lifestyle habits, we cannot rule out the possibility of confounding by such variables as socioeconomic status, which is usually associated with detrimental lifestyle habits as well as with an increased risk of cancer occurrence (Kogevinas et al., 1997). As a result, the effect of lifestyle habits in this study may have been overestimated.

Finally, the presence of biases in the estimation of the effect of the lifestyle habits cannot be discounted. Lifestyle assessment was based on self-reporting, which may have resulted in information bias by misclassification. Validity studies showed an acceptable agreement between self-reported and dietary record or health check-up measurements for alcohol consumption (correlation coefficients of 0.77 for men and 0.51 for women; results based on the first cohort) (Sasaki et al., 2003) and BMI (men, 0.89; women, 0.90; results based on the whole cohort) (Inoue et al., 2004b) and relatively low for salt consumption (men, 0.47 and 0.50; women, 0.32 and 0.31; results based on the first and second cohort respectively; unpublished results) and physical activity (men, 0.53; women, 0.35) (Inoue et al., 2008b); self-reporting of smoking status was not assessed for validity but is thought to be quite accurate. Because of the prospective nature of the study, misclassification is thought to be non-differential, and therefore it may have resulted in an underestimation of the effects of the lifestyle habits. In addition, the high response rate and the low proportion of loss to follow-up (4.4% of the study population) may have limited the risk of selection bias.

Conclusion

The present work provides insight into the repercussions of adherence to healthy lifestyle habits on the probability of developing cancer. Our results may be used by general practitioners as well as public health professionals to encourage individuals to modify potentially harmful lifestyle habits.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A

Members of the JPHC Study Group (principal investigator: S. Tsugane): S. Tsugane, M. Inoue, T. Sobue, and T. Hanaoka, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cardiovascular Center, Suita; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, and Y. Tanaba, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, and N. Nagai, Akita Prefectural Yokote Public Health Center, Yokote; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, and M. Machida, Nagano Prefectural Saku Public Health Center, Saku; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A.

Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, and T. Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito; T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Kashiwazaki and Nagaoka; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada; H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Sueta, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa; H. Sakiyama, N. Onga, H. Takaesu, and M. Uehara, Okinawa Prefectural Miyako Public Health Center, Hirara; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Suita; S. Matsushima and S. Natsukawa, Saku General Hospital, Usuda; M. Akabane, Tokyo University of Agriculture, Tokyo; M. Konishi, K. Okada, and I. Saito, Ehime University, Toon; H. Iso, Osaka University, Suita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya, Tsukuba University, Tsukuba; H. Sugimura, Hamamatsu University, Hamamatsu; Y. Tsubono, Tohoku University, Sendai; M. Kabuto, National Institute for Environmental Studies, Tsukuba; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Osaka Medical Center for Health Science and Promotion, Osaka; N. Yasuda, Kochi University, Nankoku; K. Nakamura, Niigata University, Niigata; S. Kono, Kyushu University, Fukuoka; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Takashima and M. Yoshida, Kyorin University, Mitaka; E. Maruyama, Kobe University, Kobe; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; T. Kadowaki, Tokyo University, Tokyo; M. Noda and T. Mizoue, International Medical Center of Japan, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; and H. Shimizu, Sakihae Institute, Gifu.

Appendix B. Supplementary data

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Plasma Isoflavone Concentrations Are Not Associated with Gastric Cancer Risk among Japanese Men and Women[1,2]

Azusa Hara,[3] Shizuka Sasazuki,[3]* Manami Inoue,[3] Tsutomu Miura,[4] Motoki Iwasaki,[3] Norie Sawada,[3] Taichi Shimazu,[3] Taiki Yamaji,[3] and Shoichiro Tsugane[3] for the Japan Public Health Center-Based Prospective Study Group

[3]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; and [4]Department of Health and Nutrition, Faculty of Human Life, Jin-ai University, Fukui, Japan

Abstract

The incidence of gastric cancer throughout the world is ~2–3 times higher in men than in women. Previous research suggested that isoflavones, which are structurally similar to 17β-estradiol, may prevent gastric cancer. Based on a large, population-based, prospective study, we recently reported a null association between dietary isoflavone intake and gastric cancer. However, epidemiologic studies using blood concentrations of isoflavones might better reflect the effect of isoflavones on gastric cancer carcinogenesis than dietary assessment. We therefore conducted a nested case-control study within the Japan Public Health Center-Based Prospective Study. Participants were followed-up from 1990 to 2004. Among 36,745 participants who answered the baseline questionnaire and provided blood samples, 483 gastric cancer cases matched to 483 controls were used in the analysis. ORs and 95% CIs were estimated with a conditional logistic regression model. The overall distribution of plasma isoflavone concentrations was not associated with the development of gastric cancer. Compared with groups with the lowest plasma concentrations (reference groups), the groups with the highest daidzein and genistein concentrations had adjusted ORs and 95% CIs of 1.11 (0.74–1.66; *P*-trend = 0.6) and 0.96 (0.64–1.44; *P*-trend = 0.9), respectively. The results did not change when analysis was based on sex, subsite, or histological type. We found no association of plasma isoflavone concentrations with gastric cancer risk. Our data support the previously observed null association between isoflavone intake and gastric cancer risk. J. Nutr. 143: 1293–1298, 2013.

Introduction

The incidence of gastric cancer is ~2–3 times higher in men than in women (1). This sex difference is consistent across international populations with different prevalences of environmental risk factors such as *Helicobacter pylori* infection and tobacco smoking and different dietary patterns (2,3). A possible explanation involves biological differences related to sex hormones such as estrogen (2).

Isoflavones, which are structurally similar to 17β-estradiol, have a particular affinity for the β-estrogen receptor (4) and may have the potential to prevent gastric cancer. However, we previously reported no association between dietary isoflavone intake and gastric cancer risk on the basis of data from a 5-y follow-up questionnaire given to participants in the population-based Japan Public Health Center-Based Prospective Study (JPHC Study)[5] (5).

Although FFQs can measure usual dietary habits (assuming that study participants do not change their dietary habits for long periods of time), such questionnaires are vulnerable to information bias such as memory decay, differential recall, and misclassification bias. In addition, the concentration of isoflavones in blood reflects individual differences in absorption and metabolism, in which intestinal microflora have an important role (6). Therefore, epidemiologic studies using blood concentrations of isoflavones might better reflect the effect of isoflavones on gastric cancer carcinogenesis than dietary assessment. However, only one small nested case-control study on isoflavone concentrations in blood samples has been reported (7).

Here, in a nested case-control study within a large, population-based, prospective study, we investigated the effect of plasma isoflavone concentrations on subsequent gastric cancer within a Japanese population that had substantially varied intakes of isoflavones (8).

Materials and Methods

Study population

The JPHC Study is an ongoing cohort study of cancer, cardiovascular disease, and other lifestyle-related diseases. The first group (Cohort I) of

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[5] Abbreviations used: CagA, cytotoxin-associated gene A; ICD-O-3, *International Classification of Diseases for Oncology*, 3rd edition; JPHC Study, Japan Public Health Center-Based Prospective Study; PHC, public health center.
* To whom correspondence should be addressed. E-mail: ssasazuk@ncc.go.jp.

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the study was started in 1990 and the second group (Cohort II) in 1993 (9). The study included 140,420 participants (68,722 men and 71,698 women), who were defined as inhabitants in the study areas [27 cities, towns, and villages served by 11 public health centers (PHCs)] and 40–59 y old (Cohort I) or 40–69 y old (Cohort II) at baseline. For the present analysis, we excluded 2 PHC areas (Tokyo and Osaka), because data on cancer incidence were not available in Tokyo and the study population was defined differently in Tokyo and Osaka. We thus defined 123,576 participants (61,009 men and 62,567 women) for the present study. The JPHC Study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In 1990 for Cohort I and 1993–1994 for Cohort II, participants were asked to reply to a lifestyle questionnaire that included sociodemographic characteristics, medical history, smoking and drinking habits, and diet. The FFQ included in the baseline questionnaire was previously described in detail (10). A total of 99,808 (81%) participants, 47,525 men and 52,283 women, responded to the questionnaire.

We excluded participants who self-reported cancer at baseline (n = 2136), those who were not Japanese (n = 18), and those who were later discovered to have moved away at baseline (n = 11). This left 97,644 eligible participants (46,803 men and 50,841 women), among whom 36,745 participants (38%; 13,467 men and 23,278 women) each donated 10-mL blood samples at health checkups conducted by the PHC in each area. As is customary, participants were asked to avoid consuming a meal after 17:00 h on the day before the examination. The time of either the last meal or the last drink of water or tea was recorded. The plasma and buffy layer were divided into four 1.0-mL tubes (3 tubes for plasma and 1 for the buffy layer), which were stored at −80°C. Blood was collected from 1990 to 1992 in Cohort I and from 1993 to 1995 in Cohort II.

Follow-up and identification of gastric cancer

Death and relocation. We observed study participants until 31 December 2004. The changes in residency status, including death, were identified annually through the residential registry in each area. To confirm causes of death, we used mortality data from the Ministry of Health, Labor and Welfare. Among 36,745 study participants, 4.5% moved outside the study area, 6.1% died, and 0.3% were lost to follow-up during the study period.

Cancer registry for the JPHC Study. Data on newly diagnosed cases of cancer were collected from 2 sources: active patient notification from the major hospitals in each study area and data linkage with population-based registries. Death certificate information was used as a supplementary information source. In our cancer registry system, the proportion of cases of gastric cancer for which information was available from death certificates only was 1.04%. This level of information quality was considered satisfactory for the present study.

Identification of gastric cancer and selection of controls. Cases of gastric cancer were extracted from the cancer registry for the JPHC Study on the basis of site [*International Classification of Diseases for Oncology*, 3rd edition (ICD-O-3) codes C160–169] (11). Up until the end of the study period, 512 new gastric cancer cases were identified. Until quite recently in Japan, the upper one-third of the stomach was called the "cardia" on the basis of the guidelines for gastric cancer classification (12). Because distinguishing the cardia, which is located mainly in the esophagogastric junction, from the upper one-third of the stomach seemed difficult, we combined tumors at these sites into one group for analysis (ICD-O-3 codes C160–161). A tumor located on the lower side of the stomach was classified as distal gastric cancer (ICD-O-3 codes C162–167). Subsites that were unclassifiable because the lesion was diffuse (ICD-O-3 code C168) or those with no information (ICD-O-3 code C169) were categorized as an unclassified subsite.

One of the authors (S.S.), in consultation with a pathologist, reviewed the records from each hospital and assigned histological classifications (13). Subdivisions were made as follows: differentiated type, corresponding to intestinal type in Lauren's classification (14,15), included papillary adenocarcinoma, tubular adenocarcinoma (well-differentiated type), and tubular adenocarcinoma (moderately differentiated type), and undifferentiated type, corresponding to diffuse type in Lauren's classification (14,15), included poorly differentiated adenocarcinoma, mucinous adenocarcinoma, and signet-ring cell carcinoma. Adenosquamous carcinoma, squamous cell carcinoma, carcinoid tumor, undifferentiated carcinoma, and miscellaneous were considered unclassified types. We identified 501 gastric cancer cases that were newly diagnosed between baseline and the end of the study period among participants who had returned the baseline questionnaire, reported no history of gastric cancer, and provided blood samples. For each case, one control was selected from participants with no history of gastric cancer who lived in the study area when the case was diagnosed. Each control was matched to a case for sex, age (±3 y), PHC area, blood collection date (±2 mo), and fasting time at blood collection (±5 h). The final analysis included 483 sets consisting of one case and one control (483 cases, 483 controls); 18 pairs were excluded for a technical error in isoflavone concentration measurement.

Laboratory assay

Plasma concentrations of isoflavones were analyzed by HPLC with a coulometric array detector in accordance with the modified methods of Gamache and Acworth (16). Concentrations of daidzein and genistein were determined by linear regression of the peak height for the corresponding standards and adjusted according to the recovery rate of the internal plasma standard. The calculated regression coefficients for peak height and isoflavone concentration revealed a linearity range of 0–1.0 μg/mL and the correlation coefficient exceeded 0.996 for both daidzein and genistein. Voltammetric response for the standard solution displayed CVs of 10.7 and 8.7% for intraday daidzein and genistein concentration variations, respectively. Recovery rates of daidzein and genistein in plasma samples were 84.8 and 82.1%, respectively. Detection limits were 2.70 μg/L for daidzein and 2.21 μg/L for genistein. Laboratory personnel were unaware of the case-control status when performing the analyses.

Statistical analysis

The comparison of baseline characteristics between cases and controls was evaluated by χ^2 test or 1-way ANOVA. Matched ORs and 95% CIs were calculated to indicate the relationship between isoflavone concentrations and gastric cancer risk. Multiple conditional logistic regression analyses were conducted to control for potential confounding factors, such as cigarette smoking, alcohol consumption, intake of salted fish preserves, salt intake, BMI, family history of gastric cancer, and *H. pylori* infection. Smoking status was divided into 4 groups: never smoker, past smoker, current smoker with <20 cigarettes/d, and current smoker with ≥20 cigarettes/d). Alcohol consumption was defined as drinker (≥1 d/wk) and nondrinker (<1 d/wk). BMI and the intake frequency of salted fish preserves were categorized into tertiles. Salt intake was treated as a continuous variable. Family history of gastric cancer was regarded as positive if at least one parent or sibling had gastric cancer. *H. pylori* infection was regarded as positive if the individual tested positive for antibodies against either whole *H. pylori* or cytotoxin-associated gene A (CagA).

Participants with a plasma daidzein concentration below the detection limit were defined as the reference group and the remaining participants were divided into tertiles according to the plasma daidzein concentrations in the controls by sex. Plasma concentrations of genistein were divided into quartiles according to plasma concentrations in the controls by sex.

Because adjustment for confounding factors did not significantly alter the results, only adjusted ORs are listed in the tables. The trend was assessed by assigning ordinal values for categorical variables.

All P values are 2 sided and significance was determined at the $P < 0.05$ level. We performed all statistical analyses with SAS software (ver. 9.1, SAS Institute).

Results

In baseline characteristics (**Table 1**), cases had a lower BMI and more *H. pylori* infection than controls. Other variables did not significantly differ between cases and controls.

We calculated the matched ORs and 95% CIs for developing gastric cancer in relation to plasma concentrations of isoflavones for all participants and separately for men and women (**Table 2**). We found no significant association between gastric cancer risk and the plasma concentration of either daidzein or genistein. By comparing the groups with the lowest plasma daidzein and genistein concentrations (reference groups) with the groups with the highest concentrations, we calculated adjusted ORs of 1.11 (95% CI: 0.74, 1.66; *P*-trend = 0.60) and 0.96 (95% CI: 0.64, 1.44; *P*-trend = 0.90) for all participants, 1.04 (95% CI: 0.64, 1.70; *P*-trend = 0.97) and 0.88 (95% CI: 0.54, 1.44; *P*-trend = 0.70) for men, and 1.00 (95% CI: 0.46, 2.15, *P*-trend = 0.9) and 1.09 (95% CI: 0.48, 2.47; *P*-trend = 0.80) for women, respectively. These results did not change when we estimated the ORs after excluding participants diagnosed as having gastric cancer within 3 y of baseline. In addition, dietary intakes of total energy, vegetables, fruits, fish, and green tea did not interact with any of the above results (for all interactions, $P > 0.10$). There was no significant association between total plasma isoflavones (sum of daidzein and genistein) concentrations and the risk of gastric cancer (data not shown).

When the cancers were stratified by histological type, smaller ORs were observed for undifferentiated cancer than for differentiated cancer, but the association did not reach significance. By comparing the groups with the highest plasma daidzein and genistein concentrations with the groups with the lowest concentrations (reference groups), we obtained adjusted OR values of 0.68 (95% CI: 0.30, 1.57; *P*-trend = 0.40) and 0.52 (95% CI: 0.19, 1.40; *P*-trend = 0.10) for undifferentiated cancer, respectively (**Table 3**).

When we based the analysis on distal gastric cancer, we obtained results similar to those for all gastric cancers; comparison between the lowest (reference) groups and the second, third, and fourth quartiles resulted in adjusted OR values of 1.11 (95% CI: 0.71, 1.74), 1.12 (95% CI: 0.71, 1.79), and 1.38 (95% CI: 0.85, 2.24) (*P*-trend = 0.20), respectively, for daidzein and 1.37 (95% CI: 0.86, 2.18), 1.40 (95% CI: 0.89, 2.22), and 1.21 (95% CI: 0.74, 1.97) (*P*-trend = 0.40), respectively, for genistein. There was no association for cancer in the upper one-third of the stomach (data not shown). These associations did not differ by sex (data not shown).

Our present results did not substantially change when we excluded participants who used exogenous female hormones or who were premenopausal among women (data not shown).

TABLE 1 Baseline characteristics of participants in a nested case-control study (JPHC Study)[1]

	Cases (n = 483)	Controls (n = 483)	P-difference[2]
Age, *y*	57.6 ± 7.2	57.6 ± 7.2	—
Women, %	32.9	32.9	—
Current smoker, %	35.8	30.6	0.3
Alcohol drinking ≥1 d/wk, %	51.1	50.9	0.5
BMI, *kg/m²*	23.0 ± 2.9	23.4 ± 2.8	0.04
Family history of gastric cancer, %	11.4	8.1	0.08
Salt intake, *g/d*	5.2 ± 2.6	5.1 ± 2.3	0.4
Salted fish preserves intake ≥1 d/wk, %	31.5	32.1	0.8
H. pylori infection,[3] %	98.8	89.7	<0.0001

[1] Values are means ± SDs or %. CagA, cytotoxin-associated gene A; JPHC Study, Japan Public Health Center-Based Prospective Study.
[2] χ^2 test or 1-way ANOVA.
[3] Including *H. pylori* IgG positive or CagA positive.

TABLE 2 Plasma isoflavone concentrations of cases and controls and the associations between plasma isoflavone concentrations and gastric cancer risk in the JPHC Study[1]

	Median plasma concentration		Cases	Controls	Adjusted OR
Isoflavone	Cases	Controls	(n = 483)	(n = 483)	(95% CI)
	μg/L				
Total					
Daidzein					
Lowest	0	0	158	166	1.00 (Reference)
Second	7.9	8.7	107	105	1.02 (0.71–1.48)
Third	26.1	27.4	110	105	1.04 (0.69–1.56)
Highest	70.4	68.4	108	107	1.11 (0.74–1.66)
P-trend					0.6
Genistein					
Lowest	28.8	30.5	104	121	1.00 (Reference)
Second	103.8	100.8	136	121	1.20 (0.82–1.77)
Third	219.3	211.0	135	120	1.19 (0.81–1.76)
Highest	466.1	460.2	108	121	0.96 (0.64–1.44)
P-trend					0.9
Men					
Daidzein					
Lowest	0	0	100	110	1.00 (Reference)
Second	8.2	9.3	82	71	1.12 (0.71–1.77)
Third	27.7	28.2	72	71	0.97 (0.58–1.62)
Highest	74.2	71.3	70	72	1.04 (0.64–1.70)
P-trend					0.97
Genistein					
Lowest	37.2	30.5	73	81	1.00 (Reference)
Second	113.6	103.8	89	81	1.14 (0.72–1.81)
Third	231.1	223.2	91	81	1.18 (0.73–1.91)
Highest	483.7	468.0	71	81	0.88 (0.54–1.44)
P-trend					0.7
Women					
Daidzein					
Lowest	0	0	58	56	1.00 (Reference)
Second	7.2	7.5	25	34	0.69 (0.34–1.43)
Third	25.0	25.9	38	34	1.08 (0.52–2.28)
Highest	66.1	59.7	38	35	1.00 (0.46–2.15)
P-trend					0.9
Genistein					
Lowest	18.8	28.5	31	40	1.00 (Reference)
Second	77.4	84.7	47	40	1.21 (0.54–2.69)
Third	200.7	191.9	44	39	1.32 (0.61–2.85)
Highest	404.4	396.1	37	40	1.09 (0.48–2.47)
P-trend					0.8

[1] Conditional logistic regression model was used. Matched for sex, age, study area, date of blood collection, and fasting time at blood collection. Further adjusted for cigarette smoking, alcohol consumption, intake of salted fish preserves, salt intake, BMI, family history of gastric cancer, and *H. pylori* infection. JPHC Study, Japan Public Health Center-Based Prospective Study.

Furthermore, the results did not change when we restricted cases and controls to participants with an *H. pylori* infection (data not shown).

Discussion

In this nested case-control study within a large prospective study of the Japanese population, we found that plasma concentrations of daidzein and genistein were not significantly associated with the risk of gastric cancer in either men or women. The

TABLE 3 Matched ORs of gastric cancer by histological type according to plasma concentrations of isoflavones in the JPHC Study[1]

Isoflavone	Cases (n = 483)	Controls (n = 483)	Adjusted OR (95% CI)
Differentiated type			
Daidzein			
Lowest	88	97	1.00 (Reference)
Second	64	64	0.98 (0.60–1.58)
Third	67	66	0.97 (0.57–1.64)
Highest	65	57	1.41 (0.82–2.44)
P-trend			0.3
Genistein			
Lowest	64	79	1.00 (Reference)
Second	68	64	1.18 (0.70–1.98)
Third	80	66	1.33 (0.80–2.21)
Highest	72	75	1.10 (0.66–1.84)
P-trend			0.6
Undifferentiated type			
Daidzein			
Lowest	50	47	1.00 (Reference)
Second	29	33	0.70 (0.32–1.53)
Third	34	33	0.65 (0.27–1.55)
Highest	34	34	0.68 (0.30–1.57)
P-trend			0.4
Genistein			
Lowest	28	28	1.00 (Reference)
Second	49	37	1.17 (0.53–2.57)
Third	43	49	0.51 (0.22–1.21)
Highest	27	33	0.52 (0.19–1.40)
P-trend			0.1

[1] Conditional logistic regression model was used. Matched for sex, age, study area, date of blood collection, and fasting time at blood collection. Further adjusted for cigarette smoking, alcohol consumption, intake of salted fish preserves, salt intake, BMI, family history of gastric cancer, and *H. pylori* infection. JPHC Study, Japan Public Health Center-Based Prospective Study.

results did not substantially change when we analyzed for gastric cancer by subsite or histological type.

We previously reported an association between plasma isoflavone concentrations and breast, prostate, and lung cancer risks from nested case-control studies within the JPHC Study (17–19), and for each cancer, we found results similar to those from studies using an FFQ (8,20,21). We previously observed that isoflavone intake was not associated with gastric cancer risk (5). As for other cancers, our observations in the present study are in line with those of our previous investigation, which was based on a cohort design in the same JPHC Study (5).

To our knowledge, only one nested case-control study (131 cases, 393 matched controls) has reported an inverse association between blood concentrations of isoflavones and gastric cancer risk (7). However, these results were based on a small series and the data were not analyzed by anatomic or histological site. In our relatively large population study, there was no association between plasma isoflavone concentrations and gastric cancer. We also found no significant association when the data were analyzed by anatomic location or histological subtype, although our sample size might have limited our ability to detect any small effects in stratified analysis. Further studies of a larger population are needed to confirm these findings.

The incidence of gastric cancer shows a male predominance (1). Although the mechanisms mediating this difference are unclear, a role for estrogens has been proposed (2). Estrogen receptor signaling pathways regulate important physiologic processes, such as cell growth and differentiation (22,23), and estrogen receptors have been reported in human and rodent gastric mucosa (24–26). However, the presence of estrogen receptors in normal stomach and gastric cancer tissues and their clinical importance, including the prognostic effect, have not been clearly demonstrated. In the present study, our results suggest that isoflavone concentrations have no substantial influence on gastric cancer development. On this basis, we do not think that the sex discrepancy in gastric cancer incidence can be explained by isoflavones, which have structures similar to that of 17β-estradiol. Another possible explanation for the null association between plasma isoflavone concentrations and gastric cancer risk in the present study is that the influence of established causes of gastric cancer, such as cigarette smoking (27), remained even after adjustment.

Several limitations of the study warrant mention. First, plasma isoflavone concentrations were measured only once for each individual. Although the consumption of soy foods is a personal dietary preference, the intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, in which we showed that repeated measurements of genistein intake by means of a FFQ were highly reproducible (correlation coefficient = 0.72 for a 1-y interval and 0.61 for a 5-y interval) (28,29). By comparison, plasma isoflavone concentrations may reflect short-term rather than long-term intake; isoflavones have short half-lives in blood (e.g., 7.7–9.5 h) (30) and plasma isoflavone concentrations are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates due to short-term intake, fasting time was matched in cases and controls. In addition, our study participants were restricted to those who participated in the baseline health checkup survey. We previously reported that health checkup participants had different background characteristics from nonparticipants and favorable lifestyle profiles (31). Thus, the associations between plasma isoflavone concentrations and gastric cancer risk could differ from those of the entire cohort and any generalization of our results should be done with caution.

The strengths of this study include its relatively large sample size and almost complete follow-up, because the quality of our cancer registry system during the study period was satisfactory. In addition, cases and controls were selected from the same cohort and the loss to follow-up was negligible, thereby avoiding selection bias. Moreover, the prospective study design and the estimation of ORs after exclusion of participants diagnosed as having gastric cancer within 3 y of baseline ensured that blood samples were collected before gastric cancer diagnosis, reducing the probability of reverse causality. Furthermore, we directly measured plasma isoflavone concentrations, which reflect absorption and metabolism.

In conclusion, we found no suggestion of an association of plasma isoflavone concentrations with the risk of gastric cancer.

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The authors' responsibilities were as follows: S.T. (principal investigator), M. Inoue conducted the study and managed the cancer data collection; A.H. analyzed and interpreted the data and prepared the manuscript; T.M. assayed to measure the concentration of isoflavones; and S.S., M. Iwasaki, N.S., T.S., and T.Y. helped to conduct the study. All authors read and approved the final manuscript.

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Appendix

Members of the JPHC Study Group (principal investigator: S. Tsugane): S. Tsugane, M. Inoue, T. Sobue, and T. Hanaoka, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cardiovascular Center, Suita; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, and Y. Tanaba, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, and N. Nagai, Akita Prefectural Yokote Public Health Center, Yokote; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, and M. Machida, Nagano Prefectural Saku Public Health Center, Saku; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, and T. Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito; T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka Public Health Center, Kashiwazaki and Nagaoka; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada; H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Sueta, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa; H. Sakiyama, N. Onga, H. Takaesu, and M. Uehara, Okinawa Prefectural Miyako Public Health Center, Hirara; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Suita; S. Matsushima and S. Natsukawa, Saku General Hospital, Usuda; M. Akabane, Tokyo University of Agriculture, Tokyo; M. Konishi, K. Okada, and I. Saito, Ehime University, Toon; H. Iso, Osaka University, Suita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya,

Tsukuba University, Tsukuba; H. Sugimura, Hamamatsu University, Hamamatsu; Y. Tsubono, Tohoku University, Sendai; M. Kabuto, National Institute for Environmental Studies, Tsukuba; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Osaka Medical Center for Health Science and Promotion, Osaka; N. Yasuda, Kochi University, Nankoku; K. Nakamura, Niigata University, Niigata; S. Kono, Kyushu University, Fukuoka; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Takashima and M. Yoshida, Kyorin University, Mitaka; E. Maruyama, Kobe University, Kobe; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; T. Kadowaki, Tokyo University, Tokyo; M. Noda and T. Mizoue, International Medical Center of Japan, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; and H. Shimizu, Sakihae Institute, Gifu.

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The aldehyde dehydrogenase 2 (*ALDH2*) Glu504Lys polymorphism interacts with alcohol drinking in the risk of stomach cancer

Keitaro Matsuo[1,2,*], Isao Oze[1], Satoyo Hosono[1], Hidemi Ito[1], Miki Watanabe[1], Kuka Ishioka[1,3], Seiji Ito[4], Masahiro Tajika[5], Yasushi Yatabe[6], Yasumasa Niwa[5], Kenji Yamao[7], Shigeo Nakamura[3], Kazuo Tajima[8] and Hideo Tanaka[1,2]

[1]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya 464–8681, Japan, [2]Department of Epidemiology, [3]Department of Clinical Pathophysiology and Clinical Pathology, Nagoya University Graduate School of Medicine, Nagoya 464-8550, Japan, [4]Department of Gastroenterological Surgery, [5]Department of Endoscopy, [6]Department of Pathology and Molecular Diagnostics, [7]Department of Gastroenterology, Aichi Cancer Center Hospital, Nagoya 464–8681, Japan and [8]Aichi Cancer Center Research Institute, Nagoya 464–8681, Japan

*To whom correspondence should be addressed. Tel: +81-52-762-6111; Fax: +81-52-763-5233;
Email: kmatsuo@aichi-cc.jp

The impact of alcohol on the risk of stomach cancer is controversial. Although aldehyde dehydrogenase 2 (*ALDH2*) Glu504Lys (rs671) polymorphism has a strong effect on acetaldehyde metabolism, little is known about its impact on stomach cancer risk when combined with alcohol drinking. This case-control study included a total of 697 incident stomach cancer case subjects and 1372 non-cancer control subjects who visited Aichi Cancer Center between 2001 and 2005. We estimated odds ratios (OR) and 95% confidence intervals (CI) for ALDH2 genotypes and alcohol consumption using logistic regression models after adjustment for potential confounders, including *Helicobacter pylori* infection. The ALDH2 504Lys allele was associated with the risk of stomach cancer, with adjusted ORs of 1.40 (95% CI, 1.11–1.76) for Glu/Lys and 1.73 (1.12–2.68) for Lys/Lys compared with Glu/Glu. Heavy drinking was associated with risk (OR 1.72, 1.17–2.52) after adjustment for *ALDH2* genotype and other confounders. Moreover, ORs for heavy drinking were 1.28 (0.77–2.12) for those with *ALDH2* Glu/Glu and 3.93 (1.99–5.79) for those with the *ALDH2* Lys allele relative to non-drinkers with the Glu/Glu genotype (*P* for interaction = 0.0054). In conclusion, *ALDH2* and alcohol drinking showed interaction for risk factors of stomach cancer, indicating that acetaldehyde plays a role in stomach carcinogenesis.

Introduction

Alcohol consumption is an established risk factor for cancers of the upper aero-digestive tract (UADT) (1–3), majority of them are squamous cell carcinoma. One major hypothesized mechanism behind alcohol-related carcinogenesis in the UADT is the involvement of acetaldehyde, a metabolite of ethanol. Aldehyde dehydrogenase 2 (ALDH2) is a key enzyme in acetaldehyde metabolism, and molecular epidemiologic studies in East Asia (4–11), where the functional *ALDH2* Glu504Lys (rs671) polymorphism is prevalent, have contributed to the conclusion that acetaldehyde has a substantial impact on carcinogenesis in humans as a result of its strong interaction with alcohol drinking (3).

To date, the association between alcohol consumption and gastric cancer, of which majority are adenocarcinoma, has been controversial. A recent meta-analysis showed no appreciable association of moderate alcohol drinking with stomach cancer, but it did find a suggestive association between heavy drinking and non-cardia adenocarcinoma (12). Although it has been hypothesized that acetaldehyde contributes to gastric carcinogenesis, as it does for UADT cancer (13,14), evidence for this association to date has been limited (15–18). Taken evidences of no association between esophageal adenocarcinoma risk and alcohol in mind (19,20), there may not be neither association nor interaction. Anyhow, it is worth to be evaluated in the population in which functionally validated *ALDH2* polymorphism is prevalent.

In this study, we investigated the association between *ALDH2* Glu504Lys (rs671) polymorphism and alcohol consumption and risk of stomach cancer in Japanese population.

Abbreviations: AG, atrophic gastritis; ALDH2, aldehyde dehydrogenase 2; OR, odds ratios; CI, confidence intervals; PG, pepsinogen; PY, pack years; UADT, upper aero-digestive tract.

Materials and methods

Study population

The case participants were 697 patients with no history of cancer who were histologically diagnosed with stomach cancer between January 2001 and December 2005 at Aichi Cancer Center Hospital in Japan. All participants were recruited under written informed consent within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (21–23), and all provided blood samples. Among the 697 subjects, 684 (98.1%) were histologically confirmed as adenocarcinoma. Among 684 cases, 379 were diffuse type and 305 were intestinal type.

The control subjects were 1372 first-visit outpatients during the same period who were confirmed to have no cancer and no history of neoplasms. Non-cancer status was confirmed by medical examinations, including radiographic examinations, with participants suspected of having stomach cancer first examined by physical or endoscopic inspection, and subsequently radiographically when indicated. Controls were selected randomly and were individually matched by age (±5 years) and sex (male and female) with a case-control ratio of 1:1–2. A total of 2069 participants (697 cases and 1372 controls) were included in this study. Response rate was over 95% for both case and control subjects. The study was approved by the institutional ethical committee of Aichi Cancer Center.

Information on alcohol consumption

Information on alcohol consumption was collected from first-visit outpatients aged 20–79 years using a self-administered questionnaire. Each participant was asked at the time of first visit to our hospital about their alcohol consumption before the development of the current symptoms, which made them visit our hospital. For the present analyses, lifetime alcohol consumption of various common beverages (Japanese sake, beer, shochu, whiskey and wine) was determined in terms of the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent measure of 180 ml; termed a *go*, this is standard measure in Japan and contains 23 g of ethanol. Drinking status was classified into the four categories of never drinker, light drinker (fewer than 5 days per week, fewer than 2 go per day), moderate drinker (5 or more days per week, fewer than 2 go per day) and heavy drinker (5 or more days per week, 2 or more go per day).

Evaluation of other lifestyle factors

Information on smoking status was obtained in the three categories of non-smoker, former smoker and current smoker, with former smokers defined as those who had quit smoking at least 1 year before study enrolment. Cumulative exposure to smoking was categorized into five groups by pack years (PY), the product of the number of packs of cigarettes smoked per day and the number of years of smoking, namely as never, PY < 20, PY < 40, PY < 60 and PY 60 or more. Consumption of fruits and vegetables was determined using a food frequency questionnaire, which included 43 single food items in eight frequency categories (24). The food frequency questionnaire was validated using a 3 day weighed dietary record as standard, which showed that reproducibility and validity were satisfactory (25,26). Participants were divided into three groups based on the distribution of fruit and vegetable consumption among controls (tertiles).

Assessment of Helicobacter pylori infection and atrophic gastritis

All cases were examined for plasma IgG levels for *Helicobacter pylori* (*H.pylori*) using a commercially available direct enzyme-linked immunosorbent assay

kit ('E Plate "Eiken" *H.pylori* Antibody'; Eiken Kagaku, Tokyo, Japan). This enzyme-linked immunosorbent assay kit was developed in Japan using an antigen extracted from the domestic strain in Japan and is commonly used in medical studies in this country (27,28). A positive status for *H.pylori* infection was defined as an *H.pylori* IgG antibody level >10 U/ml in serum (27,28). Serum pepsinogens (PGs) were measured by chemiluminescence enzyme immunoassay, and gastric mucosal atrophy was defined by a PG I value ≤70 ng/ml and PG I/PG II ≤ 3 ng/ml (29–31).

Examination of ALDH2 Glu504Lys (rs671) polymorphism

DNA of each subject was extracted from the buffy coat fraction using a DNA blood mini kit (Qiagen). Genotyping for the *ALDH2* Glu504Lys polymorphism (rs671) was based on TaqMan Assays by Applied Biosystems (Foster City, CA). In our laboratory, the quality of genotyping is routinely assessed statistically using the Hardy–Weinberg test and by retyping of a random sampling of 5% of subjects.

Data analyses

To assess the association between ALDH2 polymorphism and alcohol consumption in the risk of stomach cancer, we estimated the odds ratios (OR) and corresponding 95% confidence intervals (CI) using multiple logistic regression models. First, we evaluated the impact of ALDH2 polymorphism and alcohol drinking separately using all subjects. For this analysis, conditional logistic regression models included terms for cumulative exposure to smoking, fruit/vegetable intake and *H.pylori* infection. We examined a model that separately evaluated ALDH2 genotype and alcohol drinking and a second model that included both. Further, we evaluated possible effect modification by ALDH2 polymorphism on the impact of alcohol consumption; for this analysis, we used unconditional logistic regression models adjusted for the same covariates as for the overall analysis. Effect modification was assessed by the likelihood ratio test between the models with and without interaction terms between the *ALDH2* polymorphism and alcohol consumption. We defined interaction term as a product of ALDH2 polymorphism (Lys allele carrier = 1 and wild-type homozygote = 0) and alcohol consumption as a continuous variable (never = 0, low = 1, moderate = 2 and heavy = 3); therefore, degree of freedom in the tests was 1. Consistency of the interaction between *ALDH2* polymorphism and alcohol consumption was assessed by stratified analysis according to the strata of the particular covariate considered with the model including three-way interaction term among ALDH2 polymorphism, alcohol consumption and stratifying factor. Association between the combination of *ALDH2* polymorphism and alcohol consumption and atrophic gastritis (AG) was evaluated in a multivariate unconditional logistic model among control subjects. Covariates considered in the model were the same as that for stomach cancer risk, except with regard to the status of AG. Missing values for covariates were treated as dummy variables in the models. All analyses were performed using Stata SE version 11.2 (STATA Corp, College Station, TX).

Results

Demographic characteristics and selected lifestyle habits of participants are shown in Table I. Age and sex were appropriately matched. The proportion of smokers was higher in cases than in controls. Cases were exposed to a higher smoking dose than controls. Prevalence of *H.pylori* infection was 82.2% in cases and 54.2% in controls. Fruit/vegetable intake between the two groups showed no apparent marked difference (27,28).

Table II presents the association between alcohol drinking and ALDH2 rs671 polymorphism and stomach cancer. We explored three models: model 1, a crude model; model 2, a confounder-adjusted model that evaluated alcohol drinking and *ALDH2* rs671 polymorphism separately and model 3, a complete model that included alcohol drinking and *ALDH2* polymorphism together. In model 3, ORs for drinking relative to non-drinking were 1.04 (0.77–1.40) for light, 1.15 (0.82–1.61) for moderate and 1.72 (1.17–2.52) for heavy drinking, indicating a dose-dependent positive association. This association remained significant after the exclusion of former drinkers from analysis (data not shown). The association between ALDH2 rs671 polymorphism was significant in model 3, with ORs relative to Glu/Glu, the normal enzyme activity genotype, of 1.40 (1.11–1.76) for Glu/Lys, 1.73 (1.12–2.68) for Lys/Lys and 1.42 (1.13–1.79) for the Lys allele carrier after adjustment for alcohol drinking. Although smoking and *H.pylori* status are potential sources of confounding for the effect of alcohol drinking, we did not observe clear evidence of confounding between these factors and ALDH2 rs671 polymorphism.

Table I. Subject characteristics

Overall	Cases		Controls	
	No. 697	%	No. 1372	%
Sex				
Male	521	74.7	1028	74.9
Female	176	25.3	344	25.1
Age (years)				
<40	34	4.9	146	10.6
40–49	72	10.3	154	11.2
50–59	245	35.2	429	31.3
60–69	210	30.1	435	31.7
>70	136	19.5	208	15.2
Smoking status				
Never	222	31.9	538	39.2
Former	181	26	403	29.4
Current	294	42.2	430	31.3
Unknown	0	0	1	0.1
PY				
Never	222	31.9	539	39.3
<20	99	14.2	286	20.9
<40	160	23.0	272	19.8
<60	117	16.8	153	11.2
60 or more	92	13.2	113	8.2
Unknown	7	1.0	9	0.7
Alcohol consumption				
Never	228	32.7	452	32.9
Light	167	24.0	412	30.0
Moderate	159	22.8	316	23.0
Heavy	132	18.9	177	12.9
Unknown	11	1.6	15	1.1
Fruit/vegetable intake				
Lowest tertile (<114.0 g/day)	263	37.7	446	32.5
Middle tertile (<199.96 g/day)	208	29.8	445	32.4
Highest tertile (≥199.96 g/day)	209	30	445	32.4
Unknown	17	2.4	36	2.6
Family history of gastric cancer				
Yes	153	22	239	17.4
No	544	78	1133	82.6
H.pylori IgG test				
Positive	124	17.8	628	45.8
Negative	573	82.2	744	54.2
AG defined by PG testing				
Negative	262	37.6	893	128.1
Positive	434	62.3	479	68.7
Unknown	1	0.1	0	0
Histologic classification				
Diffuse	379	54.4	—	
Intestinal	305	43.8	—	
Unknown	13	1.9	—	

Table III shows results for the interaction of ALDH2 rs671 polymorphism with alcohol consumption on the risk of stomach cancer. Among ALDH2 Glu/Glu, there was no statistically significant association. In contrast, heavy drinking among ALDH2 Lys allele carriers showed a statistically significant association, with ORs among *ALDH2* Lys+ subjects of 0.79 (0.55–1.11) for light, 1.18 (0.80–1.75) for moderate and 2.37 (1.37–4.12) for heavy drinking relative to non-drinking with *ALDH2* Glu/Glu. A significant interaction between drinking and *ALDH2 Lys* allele was seen (*P*-interaction = 0.0054). We further evaluated the consistency of the gene–environment interaction between the *ALDH2* Lys allele and alcohol drinking across strata of confounders. As shown in Table IV, interaction between the two factors was consistently observed, with some exception like fruit and vegetable consumption and *H.pylori* status.

Table II. Association between ALDH2 genotype and drinking and stomach cancer risk

	Case	Control	Model 1[a]	Model 2[b]	Model 3[c]
			OR (95% CI)[b]	OR (95% CI)[b]	OR (95% CI)[b]
Level of drinking					
Non-drinker	228	452	Reference	Reference	Reference
Ever drinker					
Light	167	412	0.81 (0.63–1.04)	0.89 (0.67–1.17)	1.04 (0.77–1.40)
Moderate	159	316	1.03 (0.79–1.34)	0.92 (0.68–1.24)	1.15 (0.82–1.61)
Heavy	132	177	1.52 (1.14–2.04)	1.29 (0.92–1.80)	1.72 (1.17–2.52)
Unknown subjects	11	15			
ALDH2 genotype[d]					
Glu/Glu	310	683	Reference	Reference	Reference
Lys+	386	689	1.24 (1.03–1.49)	1.27 (1.04–1.56)	1.42 (1.13–1.79)
Glu/Lys	323	580	1.23 (1.02–1.49)	1.25 (1.01–1.54)	1.40 (1.11–1.76)
Lys/Lys	63	109	1.27 (0.91–1.78)	1.42 (0.98–2.08)	1.73 (1.12–2.68)

[a]Crude ORs by the conditional logistic regression model.
[b]ORs were calculated by a conditional logistic regression model adjusted for PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing and *H.pylori* status.
[c]ORs were calculated by unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing, *H.pylori* status, levels of drinking and ALDH2 genotypes.
[d]One case was excluded because ALDH2 genotype was not defined.

Table III. Association between ALDH2 genotype and drinking and stomach cancer risk[a]

Level of drinking	ALDH2 Glu/Glu			ALDH2 Lys+			*P*-interaction
	Case	Control	OR (95% CI)[b]	Case	Control	OR (95% CI)[b]	
Non-drinker	49	112	Reference	179	340	1.24 (0.82–1.90)	0.0054
Ever drinker							
Light	87	208	1.07 (0.67–1.70)	80	204	1.03 (0.63–1.67)	
Moderate	79	208	0.89 (0.54–1.44)	80	108	1.57 (0.94–2.64)	
Heavy	87	145	1.28 (0.77–2.12)	44	32	3.03 (1.59–5.79)	
Unknown subjects	8	10		3	5		

[a]One case was excluded because ALDH2 genotype was not defined.
[b]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atropy defined by serological PG testing and *H.pylori* status.

Table V explores the interaction between *ALDH2* genotype and alcohol drinking with regard to the prevalence of AG among non-cancer controls. Association with alcohol drinking was not significant. In analysis of the combination of *ALDH2* and alcohol drinking, heavy drinking with *ALDH2* Lys+ showed an OR of 4.50 (1.51–13.43, $P = 0.007$) relative to non-drinkers with *ALDH2* Glu/Glu, whereas that of heavy drinking with *ALDH2* Glu/Glu was 1.48 (0.74–2.98). The sources of confounding were age, sex, smoking status and *H.pylori* status.

Discussion

In this large case-control study, we found a significant interaction between the *ALDH2 Lys* allele and alcohol consumption after adjustment for *H.pylori* infection, cumulative exposure to smoking, and fruit/vegetable intake. Subjects with the *ALDH2* Lys allele who drank heavily showed a >2-fold higher risk than those with *ALDH2* Glu/Glu genotype who did not drink. A similar phenomenon was observed with regard to the prevalence of AG among non-cancer controls.

ALDH2 is a key enzyme that catalyzes acetaldehyde into acetate. The polymorphism Glu504Lys (rs671) has sufficient functional strength to influence many alcohol-related conditions (4,18,32). We first described a strong gene–environment interaction between alcohol drinking and the *ALDH2* Glu504Lys polymorphism in esophageal cancer risk (4), and subsequent studies, including our own, confirmed the same phenomenon in UADT cancers (5–11). This line of epidemiological evidence for an interaction between these two factors finally lead to the conclusion that 'acetaldehyde associated with alcoholic beverages' was Group 1 by the International Agency for Research on Cancer (3). Although the effect size of *ALDH2* or alcohol drinking was smaller than those for UADT cancers, our results are consistent with the phenomenon seen in UADT cancers, indicating the substantial attribution of acetaldehyde to stomach carcinogenesis, as previously hypothesized (13,14).

To date, several studies have evaluated the association between *ALDH2* rs671 polymorphism and risk of stomach cancer (15–18,33,34). However, these studies did not examine the interaction with detailed information on alcohol consumption. A recent study from Korea reported a similar phenomenon among 454 cases and 370 controls (17). Interestingly, a very recent study from Europe reported that a polymorphism in *ALDH2*, rs16941667, showed an allelic OR of 1.34 in a European population. But the interaction between rs16941667 and alcohol consumption is not remarkable, possibly because rs16941667 has less functional impact than rs671. In any case, their finding might indicate a substantial contribution of ALDH2 to stomach carcinogenesis across ethnicities. Clarification of the role of alcohol in gastric carcinogenesis awaits further studies of possible gene–gene interactions between the *ALDH2* and alcohol dehydrogenases genes.

In this study, we also explored the potential contribution of *ALDH2*–alcohol interaction in AG, which has been established as a pre-cancerous stage of stomach cancer (28,35,36). We defined AG status by PG I and II levels, which reflect the secretary function of gastric glands. We observed that the impact of heavy drinking was stronger in those with *ALDH2* Lys+ compared with *ALDH2* Glu/Glu, albeit that the statistical interaction was not significant. This finding might suggest that acetaldehyde plays a role in gastric carcinogenesis from the AG stage *via* induction of mutagenic adducts as reported (14) in the gastric mucosa. Against this, however, contradicting results have been reported from

Table IV. OR for heavy drinking compared with non-drinking stratified by potential confounders

Stratified by	Glu/Glu				Lys+				*P*-heterogeneity
	Non-drinker	Light	Moderate	Heavy	Non-drinker	Light	Moderate	Heavy	
	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	OR (95% CI)[a]	
Overall	Reference	1.07 (0.67–1.70)	0.89 (0.54–1.44)	1.28 (0.77–2.12)	1.24 (0.82–1.90)	1.03 (0.63–1.67)	1.57 (0.94–2.64)	3.03 (1.59 (5.79)	
Sex									
Male	Reference	1.10 (0.45–2.69)	1.04 (0.43–2.52)	1.43 (0.59–3.47)	1.42 (0.59–3.38)	1.16 (0.48–2.82)	1.85 (0.76–4.52)	3.47 (0.76–4.52)	0.823
Female	Reference	1.38 (0.72–2.67)	0.69 (0.26–1.80)	3.72 (0.52–26.7)	1.29 (0.75–2.20)	1.02 (0.43–2.41)	1.13 (0.16–7.90)	2.63 (0.16–7.90)	
Age category									
<60	Reference	0.67 (0.34–1.34)	0.68 (0.34–1.38)	1.33 (0.64–2.76)	1.29 (0.70–2.39)	1.20 (0.60–2.40)	1.32 (0.61–2.87)	1.71 (0.67–4.37)	0.751
60 or more	Reference	1.64 (0.85–3.19)	1.07 (0.54–2.14)	1.18 (0.57–2.44)	1.17 (0.65–2.12)	0.81 (0.40–1.65)	1.82 (0.89–3.70)	4.99 (1.94–12.8)	
Smoking status									
Never	Reference	1.15 (0.65–2.06)	1.05 (0.53–2.07)	1.08 (0.42–2.77)	1.16 (0.71–1.89)	1.22 (0.61–2.43)	1.66 (0.65–4.25)	2.50 (0.69–9.06)	0.187
Ever	Reference	1.10 (0.39–3.09)	0.93 (0.33–2.61)	1.63 (0.58–4.56)	1.60 (0.58–4.39)	1.12 (0.40–3.13)	1.84 (0.65–5.18)	3.89 (1.27–11.9)	
Fruit/vegetable intake									
Lowest tertile	Reference	0.45 (0.19–1.04)	0.47 (0.19–1.20)	0.51 (0.21–1.21)	0.84 (0.38–1.86)	0.48 (0.20–1.16)	0.79 (0.31–1.99)	0.95 (0.31–2.84)	0.023
Middle tertile	Reference	1.69 (0.67–4.25)	1.59 (0.63–4.06)	2.42 (0.93–6.27)	1.42 (0.61–3.27)	1.99 (0.76–5.03)	1.67 (0.62–4.51)	4.94 (1.60–15.3)	
Highest tertile	Reference	1.36 (0.62–2.95)	0.89 (0.39–2.06)	1.64 (0.60–4.51)	1.58 (0.80–3.14)	1.27 (0.56–2.91)	2.78 (1.12–6.87)	9.89 (2.16–45.3)	
H.pylori									
Positive	Reference	1.21 (0.71–2.08)	1.14 (0.65–1.98)	1.49 (0.83–2.64)	1.60 (0.8–2.61)	1.12 (0.64–1.97)	2.44 (1.35–4.42)	3.87 (1.82–8.24)	0.097
Negative	Reference	0.79 (0.30–2.10)	0.52 (0.18–1.53)	0.87 (0.29–2.57)	0.57 (0.24–1.40)	0.79 (0.29–2.14)	0.43 (0.12–1.51)	1.89 (0.50–7.11)	
AG defined by PG test									
Positive	Reference	1.00 (0.53–1.89)	1.11 (0.58–2.13)	1.18 (0.59–2.35)	1.26 (0.71–2.23)	0.92 (0.47–1.82)	1.75 (0.87–3.54)	2.35 (0.99–5.58)	0.808
Negative	Reference	1.38 (0.67–2.83)	0.73 (0.33–1.63)	1.56 (0.72–3.37)	1.46 (0.76–2.82)	1.27 (0.61–2.66)	1.84 (0.82–4.15)	5.95 (2.17–16.3)	
Family history of gastric cancer									
Yes	Reference	0.58 (0.19–1.76)	0.47 (0.14–1.54)	1.40 (0.42–4.62)	0.64 (0.23–1.73)	0.45 (0.14–1.41)	1.30 (0.40–4.25)	3.42 (0.82–14.2)	0.483
No	Reference	1.24 (0.74–2.09)	1.01 (0.59–1.73)	1.22 (0.69–2.14)	1.44 (0.90–2.31)	1.23 (0.72–2.12)	1.58 (0.88–2.84)	2.86 (1.37–5.94)	
Histology[b]									
Diffuse	Reference	1.11 (0.64–1.95)	0.97 (0.54–1.76)	1.68 (0.92–3.08)	1.50 (0.92–2.46)	1.19 (0.66–2.13)	2.00 (1.07–3.74)	3.76 (1.74–8.14)	NE[c]
Intestinal	Reference	0.89 (0.44–1.79)	0.66 (0.32–1.35)	0.82 (0.39–1.73)	0.82 (0.43–1.58)	0.66 (0.31–1.37)	1.04 (0.49–2.20)	1.96 (0.81–4.71)	
Location of stomach cancer									
Upper[d]	Reference	0.48 (0.03–8.53)	0.89 (0.07–11.7)	2.25 (0.20–25.9)	1.47 (0.15–14.3)	3.57 (0.36–35.8)	1.45 (0.10–20.3)	4.32 (0.29–64.6)	NE[c]
Others	Reference	1.09 (0.68–1.75)	0.89 (0.55–1.46)	1.26 (0.76–2.10)	1.24 (0.81–1.90)	0.98 (0.60–1.60)	1.58 (0.94–2.67)	2.89 (1.51–5.56)	

[a]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer, gastric atrophy defined by serological PG testing and *H.pylori* status.
[b]One case was excluded from analysis because of undefined histology.
[c]NE indicates not evaluable.
[d]Upper stomach cancer includes ICD O3T C16.0 (cardia, NOS, $n = 21$) and C16.1 (fundus of stomach, $n = 3$).

Table V. Associations between ALDH2 genotype and drinking and AG prevalence among controls

Level of drinking	Overall			Combined with ALDH2 genotype					
				ALDH2 Glu/Glu			ALDH2 Lys+		
	AG	Non-AG	OR (95% CI)[b]	AG	Non-AG	OR (95% CI)[b]	AG	Non-AG	OR (95% CI)[b]
Non-drinker	163	289	Reference	39	73	Reference	124	216	1.65 (0.92–2.93)
Ever drinker									
Light	128	284	0.99 (0.68–1.44)	68	140	1.71 (0.90–3.25)	60	144	1.27 (0.66–2.44)
Moderate	119	197	1.20 (0.81–1.79)	76	132	1.67 (0.88–3.17)	43	65	2.10 (1.00–4.41)
Heavy	66	111	1.19 (0.73–1.92)	51	94	1.48 (0.74–2.98)	15	17	4.50 (1.51–13.43)
Unknown subjects	3	12		1	9		2	3	

[a]One case was excluded because ALDH2 genotype was not defined.
[b]ORs were calculated by an unconditional logistic regression model adjusted for age, sex, PY of smoking, fruit/vegetable intake, family history of gastric cancer and *H.pylori* status.

Germany (37). In their population-based study in 9444 older adults, Gao *et al.* (37) found that alcohol drinking was associated with a reduced risk of AG, which they explained as due to the potentially bactericidal effect of alcohol. The attribution of *ALDH2* or alcohol consumption to gastric carcinogenesis thus remains to be elucidated.

This study had several methodological strengths. First, potential confounding by age, sex, smoking, fruit/vegetable intake, *H.pylori* infection and gastric atrophy status was considered by individual matching and statistical adjustment in the analyses. In particular, the consideration of *H.pylori* infection warrants the robustness of our observation. Second, as the *ALDH2* genotype does not change throughout life, we can assume that the impact of *ALDH2* polymorphism is subject to Mendelian randomization. Third, the size of the study was large, and the food frequency questionnaire was satisfactorily valid and reproducible (17,18). Potential limitations of this study also warrant mention. First, measurement of alcohol drinking might have been affected by the status of cases at recruitment. To avoid this, we asked about drinking behavior when the participants were healthy or before the current symptoms developed. Second, the control participants were selected from among non-cancer patients at our hospital. Because cases and controls were selected from the same hospital and almost all patients lived in the Tokai area of central Japan, the internal validity of this case-control study is likely acceptable (21). In addition, to dilute any bias that might have resulted from the inclusion of a specific diagnostic group that is related to the exposure, we did not set eligibility criteria for control diseases. Finally, it is difficult to completely rule out misclassification of *H.pylori* infection status or AG status by plasma measurement, or lifestyle factors considered as potential confounders based on self-reporting. If present, however, the effect of such misclassification in relation to possible under-adjustment would be limited, particularly considering the consistency of results across stratified analyses by several potential confounders.

In conclusion, we found that ALDH2 and alcohol drinking interact with each other in the risk of stomach cancer. This finding indicates a substantial role of acetaldehyde in carcinogenesis in the stomach, as has already been shown for cancers of the UADT.

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