Mortality from malignant tumors (SMR, 3.14; 95% CI, 1.79–5.09), especially from oropharyngeal cancer (SMR, 68.40; 95% CI, 24.98–148.88), was much higher than that in the general population. SMR of esophageal cancer was high, although it was not significantly different from that in the general population (SMR, 4.82; 95% CI, 0.06–26.81).

In subgroup A overall mortality (SMR, 0.86; 95% CI, 0.41–1.57) and mortality from malignant tumors (SMR, 1.08; 95% CI, 0.35–2.52) were similar to those of the general population (Table 3).

DISCUSSION

The present study showed that high overall mortality in patients with esophageal cancer after ER was mainly due to elevated mortality from second primary cancer. In subgroup A patients had a similar survival rate to that of the general population, which indicates complete control of esophageal cancer in almost all patients, and the invasiveness of ER did not increase the risk of serious side effects that might trigger death.

In this study, 49 of 110 patients had a present or past history of second primary cancer. Among these, 22 patients had oropharyngeal cancer. Multiple developments of squamous cell carcinoma in the esophagus and oropharynx,[16,17] frequently seen in patients with esophageal cancer, are explained by the field cancerization theory.[18] The prognosis of double primary cancers is usually influenced by oropharyngeal as well as esophageal cancer.[19,20]

Other than oropharyngeal cancer, mortality from cirrhosis (SMR, 9.73; 95% CI, 1.09–35.12) was significantly higher than that in the general population, whereas that from liver cancer (SMR, 3.34; 95% CI, 0.67–9.76) and lung cancer (SMR, 2.56; 95% CI, 0.52–7.49) was high, without being statistically significant. In the present study 5 patients died of liver-related diseases (3 with hepatic carcinoma and 2 with cirrhosis). Four of these 5 patients had hepatitis C virus (HCV) and cirrhosis at the time of ER and 3 were heavy alcohol drinkers (consumption >50 g ethanol/day). It is well known that the risk for esophageal cancer increases in proportion to the amount of alcohol consumed. A mutant allele in the ALDH gene, which is prevalent in Asians, enhances the risk of cancer.[21] Similarly, alcohol consumption is an important risk factor in the progression of HCV-related liver diseases,[22] and is regarded as a major risk factor for the rise in liver cancer mortality.[23]

Furthermore, patients with cirrhosis usually receive annual endoscopic examination for evaluation of esophageal varices. An increased opportunity

TABLE 2
Number of Patients With Local Recurrence and Metachronous Cancer After ER

	Total n = 110	Subgroup A n = 90
Complete follow-up	108	88
No. of patients with local recurrence	8	7
No. of patients with metachronous esophageal cancer after ER	12	11
No. of patients with metachronous second primary cancer after ER	15	14

ER indicates endoscopic resection.

TABLE 3
SMR in All Patients and Subgroup A

	All patients, n = 110			Subgroup A, n = 90		
	Mean follow-up, y [SD]: 4.7 [2.7]			Mean follow-up, y [SD]: 5.1 [2.6]		
	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
All deaths	22	13.07	1.68 (1.05–2.55)	10	11.69	0.86 (0.41–1.57)
Malignant tumor	16	5.10	3.14 (1.79–5.09)	5	4.62	1.08 (0.35–2.52)
Oropharynx	6	0.09	68.40 (24.98–148.88)	0	0.08	0.00 (0.00–46.88)
Liver	3	0.90	3.34 (0.67–9.76)	3	0.81	3.72 (0.75–10.88)
Esophagus	1	0.21	4.82 (0.06–26.81)	1	0.18	5.41 (0.07–30.10)
Lung	3	1.17	2.56 (0.52–7.49)	0	0.07	0.00 (0.00–3.43)
Liver cirrhosis	2	0.21	9.73 (1.09–35.12)	2	0.18	11.01 (1.24–39.73)
Circulatory diseases	4	3.38	1.18 (0.32–3.03)	3	3.02	0.99 (0.20–0.90)

SMR indicates standardized mortality ratio; CI, confidence interval.
Difference from the expected number of deaths was considered significant if 95% CI of SMR did not include unity.
Circular disease includes cerebrovascular vascular diseases and cardiovascular diseases.

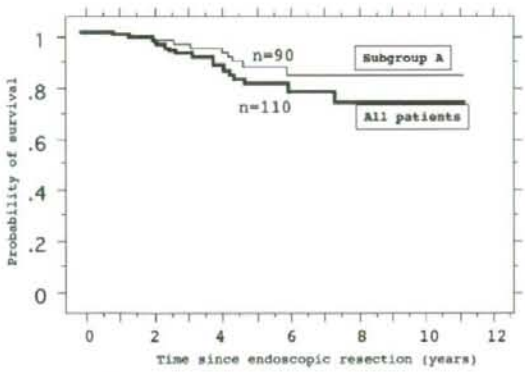

FIGURE 2. Overall survival of all patients and subgroup A.

to receive endoscopic examination enhances the discovery rate of esophageal cancer. Higher mortality from liver diseases after ER of esophageal cancer must be due to the close relation between the 2 diseases, rather than any effect of ER. A similar explanation is possible for the relatively higher mortality from lung cancer, because smoking is a major risk factor for both esophageal and lung cancer,[24] and patients with esophageal cancer have more opportunity to receive chest computed tomography.

Sato et al.[19] reported that, in patients with double primary cancers, second primary cancer is the major cause of death, as long as esophageal cancer does not have lymph node involvement. In agreement with this study, our cohort had a higher mortality rate from malignant tumors (SMR, 3.14, 95% CI, 1.79–5.09), especially from oropharyngeal cancer (SMR, 68.40; 95% CI, 24.98–148.88), and this resulted in a higher overall mortality rate. This indicates the importance of treatment of second primary cancer in patients receiving ER.

The SMR of esophageal cancer was high, although it was not significantly different (SMR, 4.82; 95% CI, 0.06–26.81) from that in the general population. The difference may have been significant if we had studied a larger number of patients. However, in this study we wanted to focus on the finding that esophageal cancer mortality, observed in only 1 case, did not negatively impact the favorable overall survival after ER.

The risk of lymph node metastasis in patients with esophageal mucosal cancer is reported to be 0% to 11%.[25–27] The higher potential for insidious metastasis in patients treated by ER results in higher recurrence and mortality. However, mucosal cancer patients without lymphovascular involvement had minimal risk of developing lymph node metastasis.[26,27] In the present study, patients with esophageal mucosal cancer and no lymphovascular involvement received no treatment after ER, whereas 3 patients with lymphovascular involvement received further treatment. Our excellent cause-specific survival after ER was achieved because patients with minimal risk for metastatic spread were accurately selected using the pathological specimens from ER.

As for long-term survival after ER, Takeshita et al.[7] have shown that there was no cause-specific death after 3 years follow-up in 43 patients with esophageal mucosal cancer treated with endoscopy. Kodama and Kakegawa[8] reported a favorable 5-year survival (>90%) after ER of esophageal mucosal cancer. Their study, although it included much information about ER, was a result of responses from 143 institutions to questionnaires on superficial cancer of the esophagus in Japan.

Makuuchi[9] reviewed the results of ER of 378 lesions in 249 patients. Lymph node metastasis and subsequent death from cancer were observed in patients who had submucosal involvement. The 5-year disease-specific survival rate of EMR was 97.9% for all patients. His report included many patients and resulted in an excellent outcome. However, detailed follow-up and cause of death were not mentioned. Sufficient follow-up is essential for survival analysis, because lower follow-up rates result in overestimation of survival.

In the present study detailed analysis of second primary cancer and follow-up data from more than 100 patients were collected with a high follow-up rate (98.2%). By analyzing SMR, overall mortality after ER was higher than that in the general population, mainly due to second primary cancer. In the subgroup analysis (patients without second primary cancer diagnosed within 1 year before ER), overall mortality after ER was similar to that in the general population, with a mean follow-up period of 5.1 years, which indicates the efficiency of ER as a curative treatment for esophageal mucosal cancer without lymphovascular involvement.

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Reduced risk of endometrial cancer from alcohol drinking in Japanese

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The role of alcohol consumption in the etiology of endometrial cancer has not been clarified. To examine the association between alcohol consumption and endometrial cancer risk, we conducted a case-control study with 148 histologically diagnosed incident endometrial cancer cases and 1468 matched non-cancer controls. Median consumption of alcohol was only 19.3 g/week among cases who drank and 28.2 g/week among controls who drank. These values are lower than in Western countries. Relative risk was analyzed in subjects classified into four groups according to weekly alcohol consumption (non-drinkers, 1–24 g/week, 25–175 g/week, and >175 g/week). Confounder-adjusted odds ratios for those consuming alcohol at <25 g/week, 25–175 g/week, and >175 g/week compared to non-drinkers were 0.79 (95% confidence interval (CI), 0.49–1.28), 0.42 (95% CI, 0.23–0.79), and 0.47 (95% CI, 0.14–1.58), respectively. Further analysis was conducted concerning self-reported physical reaction to alcohol. Among women without flushing after drinking, a significant inverse association between risk and alcohol intake was seen (trend $P = 0.001$). In contrast, no protective effect of alcohol was seen among women who experience flushing after drinking. These results suggest the presence of an inverse association between alcohol drinking and endometrial cancer risk among Japanese women, and that this association is evident among those without flushing. Further investigation of these findings is warranted. (*Cancer Sci* 2008; 99: 1195–1201)

Endometrial cancer is a common gynecologic cancer in Japan, and its incidence is increasing, possibly due to the recent Westernization of the Japanese lifestyle.[1] The development of endometrial cancer has been related to exposure to unopposed estrogens.[2–4] Several studies have shown a positive association between alcohol intake and estrogen level in postmenopausal women.[5,6] Although alcohol intake could therefore be expected to increase the risk of endometrial cancer by elevating estrogen levels, epidemiologic studies of this association have been inconsistent. Most previous studies have indicated that alcohol consumption is either weakly or not associated with the risk of endometrial cancer.[7–11] However, several others have shown an increased risk in heavy drinkers[12,13] while a case-control study by Swanson *et al.* suggested an inverse association between moderate alcohol consumption and endometrial cancer risk among young women (<55 years).[14] These inconsistent findings, as well as uncertainties regarding the etiology of endometrial cancer, hamper any coherent understanding of this association.

Here, we conducted a hospital-based case-control study to examine the association between alcohol consumption and endometrial cancer risk among Japanese women, considering other predisposing characteristics, such as body mass index and a history of hormone replacement therapy. In addition, given recent findings that a genetic polymorphism in *aldehyde dehydrogenase2 (ALDH2)*, which has a strong impact on alcohol metabolism, was associated with several cancer risks,[15–17] we also analyzed this risk using self-reported reactions after drinking as a surrogate for *ALDH2* genotyping.

Materials and Methods

Subjects. The subjects were 148 patients newly and histologically diagnosed with endometrial carcinoma between January 2001 and June 2005 at Aichi Cancer Center Hospital (ACCH) in Japan. The distribution of histological subtypes among 148 cases was 93 type I tumor (low-grade endometrioid adenocarcinoma) (62.8%), and 55 type II tumor (high-grade endometrioid adenocarcinoma and other adenocarcinomas) (37.2%). Mixed epithelial and mesenchymal tumors were excluded due to the paucity of knowledge on their etiology. Controls ($n = 1476$) were randomly selected and matched by age (± 3 years) and menopausal status (premenopause or postmenopause) to cases with a 1:10 case-control ratio from 11 814 women who were diagnosed as cancer-free (four cases were matched with nine controls). All subjects were recruited in the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), as described elsewhere.[18,19] In brief, information on lifestyle factors was collected using a self-administered questionnaire for all first-visit outpatients at Aichi Cancer Center Hospital aged 20–79 who were enrolled in HERPACC between January 2001 and November 2005. Patients were also asked about lifestyle when healthy or before the current symptoms developed. Responses were checked by a trained interviewer. Approximately 90% of eligible subjects completed the questionnaire. Outpatients were also asked to provide blood samples. Our previous study showed that the lifestyle patterns of first-visit outpatients accorded with those in a randomly selected sample of the general population of Nagoya City.[20] The data were loaded into the HERPACC database and routinely linked with the hospital-based cancer registry system to update the data on cancer incidence. All participants gave written informed consent and the study was approved by Institutional Ethical Committee of Aichi Cancer Center.

Assessment of alcohol intake and alcohol reaction. All subjects were asked about their average frequency, beverage type, and amount of drinking per day during the 1-year period before onset of the present disease or before being interviewed. Usual alcohol intake was first reported as frequency of consumption in the five categories of non-drinker, <1 day/week, 1–2 days/week, 3–4 days/week, and 5 or more days per week. Consumption of each type of beverage (Japanese sake, beer, shochu, whiskey,

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Table 1. Characteristics of subjects

Characteristic	Cases		Controls		*P*-values
Number	148		1476		
Age (median, [min–max])	56.0 (26–79)		56.0 (23–80)		0.846
≤39 (%)	22	(14.9)	223	(15.1)	0.986
40–49 (%)	13	(8.8)	136	(9.2)	
50–59 (%)	64	(43.2)	610	(41.3)	
60–69 (%)	36	(24.3)	385	(26.1)	
≥70 (%)	13	(8.8)	122	(8.3)	
Smoking status					
Ever (%)	24	(16.2)	244	(16.5)	0.942
Never (%)	123	(83.1)	1225	(83.0)	
Unknown (%)	1	(0.7)	7	(0.5)	
Body mass index (median, [min–max])	23.2 (13.4–40.9)		21.9 (13.2–42.7)		<0.001
<25 kg/m² (%)	104	(70.3)	1211	(82.1)	<0.001
≥25 kg/m² (%)	40	(27.0)	257	(17.4)	
Unknown (%)	4	(2.7)	8	(0.5)	
Regular exercise					
No (%)	46	(31.1)	388	(26.3)	0.252
Yes (%)	101	(68.2)	1057	(71.6)	
Unknown (%)	1	(0.7)	31	(2.1)	
Menstrual status					
Premenopausal (%)	51	(34.5)	506	(34.3)	0.965
Postmenopausal (%)	97	(65.5)	970	(65.7)	
Age at menarche (median, [min–max])	14.0 (10–20)		14.0 (10–21)		0.963
≤12 (%)	38	(25.7)	379	(25.7)	0.729
13–14 (%)	75	(50.7)	701	(47.5)	
≥15 (%)	31	(21.0)	365	(24.7)	
Unknown (%)	4	(2.7)	31	(2.1)	
Duration of menstration (median, [min–max])	37.0 (0–49)		36.0 (11–43)		0.390
≤32 (%)	38	(25.7)	395	(26.8)	0.822
33–36 (%)	33	(22.3)	367	(24.9)	
37–39 (%)	38	(25.7)	388	(26.3)	
≥40 (%)	34	(23.0)	284	(19.2)	
Unknown (%)	5	(3.4)	42	(2.9)	
Parity (median, [min–max])	2 (0–4)		2 (0–6)		<0.001
0 (%)	41	(27.7)	207	(14.0)	<0.001
1–2 (%)	82	(55.4)	911	(61.7)	
≥3 (%)	24	(16.2)	348	(23.6)	
Unknown (%)	1	(0.7)	10	(0.7)	
Diabetes history					
No (%)	137	(92.6)	1416	(95.9)	0.056
Yes (%)	11	(7.4)	60	(4.1)	
Hypertension history					
No (%)	121	(81.8)	1273	(86.3)	0.135
Yes (%)	27	(18.2)	203	(13.8)	
Contraceptive usage history					
No (%)	138	(93.2)	1377	(93.3)	0.934
Yes (%)	8	(5.4)	74	(5.0)	
Unknown (%)	2	(1.4)	25	(1.7)	
Hormone replacement therapy history					
No (%)	132	(89.2)	1355	(91.8)	0.247
Yes (%)	15	(10.1)	100	(6.8)	
Unknown (%)	1	(0.7)	21	(1.4)	

and wine) was determined by the average number of drinks per day, which was then converted into a Japanese sake (rice wine) equivalent. One Japanese drink equates to one 'go' (180 mL) of Japanese sake, which contains 23g of ethanol, equivalent to one large bottle (633 mL) of beer, two shots (57 mL) of whiskey, or 2.5 glasses of wine (200 mL). One drink of shochu (distilled spirit), which contains 25% ethanol, was rated as 108 mL. Total alcohol consumption was estimated as the summed amount of pure alcohol consumption (g/drink) of Japanese sake, beer, shochu, whiskey, and wine among current regular drinkers. Weekly ethanol consumption was calculated by combining the amount of ethanol per day and frequency per week. In this study, we used self-reported flushing (yes/no) after a small amount of drinking (a glass of beer) as a stratification factor in the examination of alcohol impact.

Statistical analysis. To assess the strength of associations between alcohol consumption and risk of endometrial cancer, odd ratios (OR) with 95% confidence intervals (CI) were estimated using unconditional logistic models adjusted for potential confounders. For subgroup analysis, subjects were classified by

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Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for endometrial cancer according to frequency and quantitiy of alcohol intake

Category	Cases (n = 148)	Controls (n = 1476)	Age-adjusted OR (95% CI)	Multivariate OR (95% CI)†
Frequency of alcohol intake				
None	108	929	1.00 (Reference)	1.00 (Reference)
<1/week	14	166	0.72 (0.40–1.29)	0.71 (0.39–1.29)
1–2/week	11	119	0.79 (0.41–1.52)	0.77 (0.40–1.50)
3–4/week	8	99	0.69 (0.33–1.46)	0.67 (0.31–1.43)
5-/week	7	154	0.39 (0.18–0.85)	0.37 (0.17–0.82)
unknown	0	9		
P-trends			0.011	0.009
Amount of alcohol consumption				
None	109	933	1.00 (Reference)	1.00 (Reference)
<25 g/week	23	246	0.79 (0.49–1.27)	0.79 (0.49–1.28)
(median, range) (eta g/week)	(8.6, 2.9–24.2)	(8.6, 1.7–24.2)		
25–175 g/week	12	232	0.44 (0.24–0.81)	0.42 (0.23–0.79)
(median, range) (eta g/week)	(54.3, 25.9–96.6)	(69, 25.3–172.5)		
>175 g/week	3	47	0.54 (0.16–1.76)	0.47 (0.14–1.58)
(median, range) (eta g/week)	(201.3, 179.4–552)	(276, 177.1–805)		
unknown	1	18		
P-trends			0.006	0.005

†Multivariate models adjusted for age, smoking, body mass index, regular exercise, menstrual status, age at menarche, duration of menstruation, parity, diabetes history, hypertension history, contraceptive usage history, hormone replacement therapy, and flushing after drinking.

alcohol intake into the four groups of non-drinkers, and weekly ethanol intake of 1–24, 25–175, and >175 g. Among controls, median weekly intake in current drinkers was 25 g. Potential confounders considered in the multivariate analyses were age, smoking habit (never smokers or ever smokers), body mass index (BMI; <25 or ≥25 kg/m^2 based upon our previous study),[21] regular exercise (yes or no), menstrual status (premenopausal or postmenopausal), age at menarche (≤ 12, 13–14, or ≥ 15), duration of menstruation (years, quartiles), parity (0, 1–2, ≥ 3), diabetes history (yes or no), hypertension history (yes or no), contraceptive usage history (yes or no), hormone replacement therapy history (yes or no), flushing after drinking (yes or no), and histological subtype (type I or type II). Missing values for any covariate were treated as a dummy variable in the logistic model. Differences in categorized demographic variables between the cases and controls were tested by the χ^2-test. Age, age at menarche, duration of menstruation, BMI, and parity between cases and controls were compared by the Mann–Whitney test. Stratification analysis was used to estimate risk for subgroups by drinking habit. *P*-values less than 0.05 were considered statistically significant. All analyses were conducted using STATA version 9 (Stata, College Station, TX, USA).

Results

Baseline characteristics of the 148 endometrial cancer patients and 1476 controls are shown in Table 1. Median age was 56 years for both patients and controls. Smoking status did not differ between the two groups. Prevalence of ever smokers was 16.2% and 16.5% in case and controls, respectively. BMI was higher among cases than controls ($P < 0.001$). Regarding reproductive factors, only parity showed a significant difference between two groups. Low experience of delivery was more prevalent among cases than controls ($P < 0.001$). A history of diabetes was more common in cases, although with only marginal statistical significance. Although contraceptive usage did not differ, hormone replacement therapy was more prevalent in cases.

Median consumption of alcohol among cases and controls who drank was only 19.3 and 28.2 g/week, respectively. Table 2 shows the impact of drinking habit on endometrial cancer risk. Frequent drinkers showed a reduced risk: compared with non-drinkers, the age-adjusted OR of those who drank 5 or more days per week was 0.39 (95% CI, 0.18–0.85). Although without significance, all groups except non-drinkers showed OR below unity and their point estimates decreased as frequency increased (P-trend = 0.011). This trend was consistently observed in the multivariate model. Similarly, with regard to the amount of alcohol consumed, those who consumed less than 25 g per week, those who consumed 25–175 g per week, and those who consumed 175 g or more per week showed a lower risk of endometrial cancer than non-drinkers, with OR of 0.79 (95% CI, 0.49–1.27), 0.44 (95% CI, 0.24–0.81), and 0.54 (95% CI, 0.16–1.76), respectively. The multivariate model again showed consistent results.

Table 3 shows a stratified analysis according to potential confounders designed to examine the consistency of association and to explore the possible interaction with weekly alcohol consumption. The inverse association between endometrial cancer risk and alcohol intake persisted after stratification by BMI, regular exercise, menstrual status, age at menarche, duration of menstruation, parity, diabetes history, hypertension history, and type I tumor. In contrast, no associations were seen for ever smokers, oral contraceptive users, hormone replacement therapy users, and type II tumor. Regarding BMI, obese women (BMI ≥ 25) showed a stronger protective effect by alcohol than leaner women (BMI < 25). Among postmenopausal women, the OR for weekly drinking of less than 25, 25–175, and 175 g or more for EC were 0.83 (95% CI, 0.46–1.52), 0.46 (95% CI, 0.21–1.02), and 0.72 (95% CI, 0.17–3.15), respectively, but the P-trend was marginally significant ($P = 0.069$). Generally, endometrial cancer risk was lowest among women with weekly consumption of 25–175 g.

Table 4 shows a stratified analysis according to self-reported reaction to alcohol. Flushing after drinking depends mainly on the activity of aldehyde dehydrogenase, particularly ALDH2, and might therefore reflect lower ALDH2 activity. Among women who did not experience flushing after drinking, an inverse association was seen between endometrial cancer risk and alcohol intake. The age-adjusted OR for weekly drinking of less than 25, 25–175, and 175 g or more for endometrial cancer were 0.51 (95% CI, 0.26–0.98), 0.24 (95% CI, 0.11–0.56), and 0.49 (95% CI, 0.14–1.69), respectively, and the P-trend was statistically significant ($P = 0.001$). By contrast, the protective

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for endometrial cancer stratified according to weekly alcohol consumption and lifestyle factors

Category	Alcohol consumption				
	None	<25 g/week	25–175 g/week	>175 g/week	*P*-trends
Total (case/control)*	109/933	23/246	12/232	3/47	
OR (95% CI)	1.00 (Reference)	0.79 (0.49–1.27)	0.44 (0.24–0.81)	0.54 (0.16–1.76)	0.006
Smoking					
Never (case/control)	98/829	18/213	5/157	1/16	
OR (95% CI)	1.00 (Reference)	0.70 (0.41–1.18)	0.26 (0.11–0.66)	0.51 (0.07–3.87)	0.002
Ever (case/cotrol)	11/98	4/33	7/75	2/31	
OR (95% CI)	1.00 (Reference)	1.25 (0.36–4.40)	0.89 (0.33–2.46)	0.63 (0.13–3.04)	0.586
Unknown (case/cotrol)	0/6	1/0	0/0	0/0	
Body mass index					
<25 kg/m² (case/control)	73/757	17/197	11/202	2/40	
OR (95% CI)	1.00 (Reference)	0.92 (0.53–1.61)	0.58 (0.30–1.12)	0.54 (0.13–2.31)	0.090
≥25 kg/m² (case/control)	32/168	6/49	1/30	1/7	
OR (95% CI)	1.00 (Reference)	0.55 (0.21–1.43)	0.15 (0.02–1.13)	0.48 (0.05–4.34)	0.035
Unknown (case/control)	4/8	0/0	0/0	0/0	
Regular exercise					
No (case/control)	36/257	7/40	2/63	1/22	
OR (95% CI)	1.00 (Reference)	1.27 (0.53–3.05)	0.23 (0.05–0.97)	0.34 (0.04–2.57)	0.047
Yes (case/control)	72/654	16/201	10/167	2/25	
OR (95% CI)	1.00 (Reference)	0.70 (0.40–1.24)	0.53 (0.27–1.05)	0.69 (0.16–3.00)	0.053
Unknown (case/control)	1/22	0/5	0/2	0/0	
Menstrual status					
Premenopausal (case/control)	35/280	9/99	5/98	1/23	
OR (95% CI)	1.00 (Reference)	0.72 (0.34–1.57)	0.41 (0.15–1.07)	0.35 (0.05–2.65)	0.038
Postmenopausal (case/control)	74/653	14/147	7/134	2/24	
OR (95% CI)	1.00 (Reference)	0.83 (0.46–1.52)	0.46 (0.21–1.02)	0.72 (0.17–3.15)	0.069
Age at menarche					
≤12 (case/control)	28/236	8/61	1/64	1/13	
OR (95% CI)	1.00 (Reference)	1.04 (0.45–2.40)	0.12 (0.02–0.92)	0.56 (0.07–4.49)	0.053
13–14 (case/control)	53/428	11/127	9/114	1/22	
OR (95% CI)	1.00 (Reference)	0.72 (0.36–1.42)	0.65 (0.31–1.37)	0.38 (0.05–2.90)	0.120
≥15 (case/control)	26/249	2/54	2/48	1/11	
OR (95% CI)	1.00 (Reference)	0.39 (0.09–1.73)	0.44 (0.10–1.91)	1.07 (0.13–8.88)	0.260
Unknown (case/control)	2/20	2/4	0/6	1/0	
Duration of menstruation					
≤32 years (case/control)	27/219	7/77	4/71	0/22	
OR (95% CI)	1.00 (Reference)	0.69 (0.28–1.67)	0.43 (0.15–1.29)	NE	0.029
33–36 years (case/control)	27/246	5/51	1/54	0/9	
OR (95% CI)	1.00 (Reference)	0.93 (0.34–2.55)	0.18 (0.02–1.35)	NE	0.063
37–39 years (case/control)	29/249	3/71	4/57	1/8	
OR (95% CI)	1.00 (Reference)	0.36 (0.11–1.23)	0.60 (0.20–1.78)	1.07 (0.13–8.88)	0.249
≥40 years (case/control)	23/189	6/43	3/43	2/7	
OR (95% CI)	1.00 (Reference)	1.13 (0.43–2.95)	0.56 (0.16–1.95)	2.23 (0.43–11.49)	0.932
Unknown (case/control)	3/30	2/4	0/7	0/1	
Parity					
0 (case/control)	30/115	6/36	4/42	1/10	
OR (95% CI)	1.00 (Reference)	0.63 (0.24–1.65)	0.36 (0.12–1.09)	0.38 (0.05–3.10)	0.046
1–2 (case/control)	58/599	15/147	6/129	2/25	
OR (95% CI)	1.00 (Reference)	1.12 (0.61–2.05)	0.50 (0.21–1.20)	0.90 (0.21–3.93)	0.271
≥3 (case/control)	21/213	2/61	1/59	0/12	
OR (95% CI)	1.00 (Reference)	0.37 (0.08–1.64)	0.19 (0.02–1.43)	NE	0.035
Unknown (case/control)	0/6	0/2	1/2	0/0	
Diabetes history					
No (case/control)	99/894	22/237	12/224	3/45	
OR (95% CI)	1.00 (Reference)	0.81 (0.50–132)	0.47 (0.25–0.87)	0.57 (0.17–1.89)	0.015
Yes (case/control)	10/39	1/9	0/8	0/2	
OR (95% CI)	1.00 (Reference)	0.48 (0.05–4.33)	NE	NE	0.212
Hypertension history					
No (case/control)	87/797	21/225	10/200	2/38	
OR (95% CI)	1.00 (Reference)	0.85 (0.51–1.40)	0.45 (0.23–0.89)	0.47 (0.11–2.00)	0.016
Yes (case/control)	22/136	2/21	2/32	1/9	
OR (95% CI)	1.00 (Reference)	0.54 (0.12–2.47)	0.36 (0.08–1.62)	0.64 (0.08–5.32)	0.178

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Table 3 (*Continued.*)

Category	Alcohol consumption				
	None	<25 g/week	25–175 g/week	>175 g/week	*P*-trends
Contraceptive usage history					
No (case/control)	101/871	23/231	12/216	1/43	
OR (95% CI)	1.00 (Reference)	0.85 (0.53–1.38)	0.47 (0.26–0.88)	0.20 (0.03–1.45)	0.005
Yes (case/control)	6/44	0/11	0/15	2/4	
OR (95% CI)	1.00 (Reference)	NE	NE	3.63 (0.53–24.92)	0.892
Unknown (case/control)	2/18	0/4	0/1	0/0	
Hormone replacement therapy history					
No (case/control)	101/860	18/227	10/212	2/40	
OR (95% CI)	1.00 (Reference)	0.66 (0.39–1.12)	0.39 (0.20–0.77)	0.41 (0.10–1.72)	0.002
Yes (case/control)	7/59	5/15	2/19	1/7	
OR (95% CI)	1.00 (Reference)	2.79 (0.78–10.05)	0.89 (0.17–4.64)	1.21 (0.13–11.31)	0.826
Unknown (case/control)	1/14	0/4	0/1	0/0	
Histological subtype					
Type I (case/control)	68/933	17/246	6/232	1/47	
OR (95% CI)	1.00 (Reference)	0.71 (0.51–1.57)	0.34 (0.14–0.79)	0.27 (0.04–1.97)	0.007
Type II (case/control)	41/933	6/246	6/246	2/47	
OR (95% CI)	1.00 (Reference)	0.60 (0.25–1.43)	0.63 (0.26–1.50)	1.09 (0.25–4.69)	0.323

[†]One case and 18 controls were excluded from analyses due to lack of information on alcohol drinking.
NE, not estimated because of no case in this category.

Table 4. Impact of alcohol consumption according to self-reported reaction to alcohol

Category	Alcohol consumption				
	None	<25 g/week	25–175 g/week	>175 g/week	*P*-trends
Total (case/control)[†]	109/933	23/246	12/232	3/47	
Age-adjusted OR (95% CI)	1.00 (Reference)	0.79 (0.49–1.27)	0.44 (0.24–0.82)	0.54 (0.16–1.76)	0.006
Multivariate OR (95% CI)	1.00 (Reference)	0.79 (0.49–1.28)	0.42 (0.23–0.79)	0.47 (0.14–1.58)	0.005
Flushing after drinking					
No (case/control)	44/292	13/157	7/175	3/36	
Age-adjusted OR (95% CI)	1.00 (Reference)	0.51 (0.26–0.98)	0.24 (0.11–0.56)	0.49 (0.14–1.69)	0.001
Multivariate OR (95% CI)	1.00 (Reference)	0.53 (0.27–1.05)	0.25 (0.11–0.59)	0.48 (0.14–1.67)	0.002
Yes (case/control)	61/574	9/86	5/55	0/10	
Age-adjusted OR (95% CI)	1.00 (Reference)	1.03 (0.49–2.15)	0.89 (0.34–2.30)	NE	0.560
Multivariate OR (95% CI)[‡]	1.00 (Reference)	1.07 (0.51–2.27)	0.97 (0.37–2.57)	NE	0.677
Unknown (case/control)	4/67	1/3	0/2	0/1	

[†]One case and 18 controls were excluded from analyzes due to lack of information on alcohol drinking.
[‡]Multivariate models adjusted for age, smoking, body mass index, regular exercise, menstrual status, age at menarche, duration of menstruation, parity, diabetes history, hypertension history, contraceptive usage history, and hormone replacement therapy.
CI, confidence interval; NE, not estimated because of no case in this category; OR, odds ratio.

effect of alcohol was not observed among women who had flushing after drinking (age-adjusted *P*-trend = 0.560). The multivariate model again showed consistent results.

Discussion

In this study, we found that a small amount of alcohol consumption was protective against endometrial cancer among Japanese women. This association was consistently observed regardless of potential confounders. OR were lowest among those who consumed 25–175 g per week. In addition, the protective effect of alcohol drinking decreased among women who reported flushing after drinking.

Results to date regarding the relationship between alcohol intake and endometrial cancer risk are inconsistent. Although most previous studies have indicated a null association,[7–9,11,22–25] three have shown a protective effect of alcohol,[10,14,26] while three others have reported that alcohol intake was a risk factor of endometrial cancer.[12,13,27] Newcomb *et al.* suggested a significant inverse association in premenopausal women consuming one drink per day or more (RR = 0.20; 95% CI, 0.06–0.71)[10] while Swanson *et al.* showed an inverse association between moderate consumption and endometrial cancer risk among young women (<55 years), with relative risks for three levels of drinking (<1, 1–4, >4 drinks per week) from lowest to highest of 0.78, 0.64, and 0.41 compared to non-drinkers.[14] Webster *et al.* showed that non-drinkers aged 20–54 years had a higher relative risk (RR = 1.83; 95% CI, 1.11–3.01) than women who consumed an average of 150 g or more of alcohol per week.[26] These results may indicate that light alcohol consumption decreases endometrial cancer risk in younger women. In contrast, Setiawan *et al.* suggested that alcohol consumption equivalent to two or more drinks per day increased the risk of endometrial cancer in postmenopausal women.[12] The other two case-control studies showed similar positive associations between increased alcohol consumption and risk.[13,27]

Here, our study has added to the evidence for a protective effect of alcohol on endometrial cancer. The degree of consumption

may be an important consideration in determining the impact of alcohol. Average consumption in our study was very low compared with previous studies. Relatively high consumption (≥175 g/week) was seen in only three cases and 99 controls, who showed a protective effect compared with non-drinkers (multivariate OR = 0.47; 95% CI, 0.14–1.58). The provision of stable estimates for this subgroup is hampered by their small sample size.

One possible explanation for these results is that a small amount of drinking might be protective against cancer, as suggested in several prospective cohort studies.[28–32] The biological mechanism of this protective effect for cancer among light-moderate drinkers is not clear. Tsugane *et al.* considered the background characteristics of moderate drinkers to be healthier than those of either non-drinkers or heavy drinkers.[32] It has been reported that alcohol intake increases endogenous serum levels of estrogen in postmenopausal women,[5,6] but it is unclear whether this is due to either a decrease in metabolic clearance or an increase in production.[33] It has thus been hypothesized that alcohol drinking might lead to an increased risk of endometrial cancer risk due via the increased mitotic proliferation of endometrial cells, resulting in increased DNA replication errors and somatic mutations.[34] Our findings here contradict this hypothesized mechanism; nevertheless, we assume that the amount of drinking may differentiate the impact of alcohol on endometrial cancer risk, as stated above.

Of interest was the combined effect of the amount of consumption and physical reaction to alcohol.[19] Subjects who reported flushing did not show the protective effect observed in the non-flushing group. It has been suspected that the oxidative metabolite of ethanol, acetaldehyde, is carcinogenic for humans due to its binding to cellular proteins and DNA, thus leading to carcinogenesis.[35,36] Further, in individuals with ALDH2 encoded by *ALDH2* Glu/Lys, the blood acetaldehyde level after drinking is approximately six-fold that in individuals with active ALDH2.[37] Taking results from our previous study demonstrating sensitivity and specificity of self-reported flushing for ALDH2 genotype as 83.5% and 87.8%,[38] our findings may have resulted from a decrease in the protective effect of alcohol owing to exposure to high levels of acetaldehyde.

Several potential limitations of our study warrant consideration. First, because it was a hospital-based case-control study, the threat of inadequate comparability between cases and controls rested on whether the control population was the source population from which cases arose. In the ACCH, it is assumed that those who are diagnosed as not having cancer at a particular period of time will visit the ACCH in the event that they do develop malignant disease. Our source of controls is therefore assumed to be appropriate for the drawing of causal inferences. Second, as with other case-control studies, this study may have suffered from recall bias. Although the questionnaires, including that on alcohol intake, were completed before diagnosis in our hospital, some case patients referred to the hospital might have known their diagnosis. The fact that alcohol intake is not a well-accepted risk factor for endometrial cancer among the public might preclude this possibility of information bias regarding alcohol Third, our study had a modest sample size, and replication in other studies is required.

In conclusion, our case-control study suggested that alcohol drinking decreases the risk of endometrial cancer among Japanese women who consume small amounts. Further, a similar association was observed after stratification by potential confounders. However, this protective effect of alcohol was modified in those who experienced a flushed reaction to it after drinking. Further investigation of these findings is warranted.

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Effect of soybean on breast cancer according to receptor status: A case–control study in Japan

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The possible association of high soy food consumption with low incidence of breast cancer in Asian countries has been widely investigated, but findings from epidemiologic studies have been inconsistent. Breast cancers defined by receptor status, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) may have distinct etiologic factors. Here, we conducted a case–control study to clarify associations between intake of soybean products and breast cancer risk according to receptor status. A total of 678 breast cancer cases and 3,390 age- and menopausal status-matched noncancer controls were included. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated using conditional logistic models adjusted for potential confounders. On analysis according to receptor status, we observed a significantly reduced risk of ER-positive (ER+) (top tertile OR = 0.74; 95% CI, 0.58–0.94; trend p = 0.01) and HER2-negative (HER2−) tumors (top tertile OR = 0.78; 95% CI, 0.61–0.99; trend p = 0.04). Further, when the 3 receptors were jointly examined, a reduced risk was observed only in patients with ER+/PR+/HER2− tumor (top tertile OR = 0.73; 95% CI, 0.54–0.97; trend p = 0.03). These findings indicate that the protective effect of soy against breast cancer risk differs by receptor status.

Key words: breast cancer; soybean; hormone receptors; HER2

Although the incidence of breast cancer in Japan has increased steadily over the last 30 years,[1] it nevertheless remains substantially lower than in Western countries.[2] Considerable interest has thus been expressed in identifying factors in the Japanese life style that modify the risk of breast cancer in this population.

Soy foods are rich in isoflavones, compounds that have been shown to exert anticarcinogenic effects on hormone-related cancers in a large number of experimental studies and have been hypothesized to reduce the risk of the cancers. In Japan, a wide variety of soy foods is available, and isoflavone consumption is consequently habitual and high. While this high consumption may account for some of the international differences in incidence, a protective effect of soybean or isoflavones against breast cancer has not been consistently found.[3]

The behavior of breast tumors is partly determined, to some extent at least, by gene expression in breast cancer tissues, such as of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2). Clinically, ER, PR and HER2 levels in tumors are used as prognostic indicators of disease course and response to adjuvant therapy. In general, the presence of ER-positive (ER+) or PR+ breast tumors, either singly or together, has been associated with better survival and overall outcome, whereas tumors with HER2 overexpression are characterized by a poor prognosis.[4] Etiologic factors related to the risk of developing breast cancer may also differ according to receptor status. Previous studies reported that reproductive factors are more strongly linked to the risk of ER+/PR+ than receptor-negative breast cancer.[5–7] Results for HER2 status, in contrast, have been inconsistent.[7–10]

Classification by receptor status may help clarify the inconclusive results for soybean consumption and risk of breast cancer. Here, to evaluate the association between soy food intake and breast cancer risk by receptor status, we conducted a case–control study using data from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC).

Material and methods

Study population

Details of the HERPACC have been described elsewhere.[11,12] In brief, HERPACC was initiated in Aichi Cancer Center Hospital, Nagoya, Japan, in 1988, with information on lifestyle factors collected from all first-visit outpatients using a self-administered questionnaire, with responses checked by a trained interviewer. Patients were asked about their lifestyle when healthy or before the current symptoms developed. Information from the questionnaire was systematically collected and checked by trained interviewers, and completed by 96.7% of 29,538 eligible subjects (2001–2005). Questionnaire data were loaded into the HERPACC database and periodically linked with the hospital cancer registry system to update data on cancer incidence. All participants gave written informed consent and the study was approved by the Ethics Committee of Aichi Cancer Center.

Ascertainment of breast cancer cases and controls

A total of 838 breast cancer patients who underwent surgical excision at the Department of Breast Oncology Aichi Cancer Center Hospital between 2003 and 2005 were deemed eligible as case subjects. ER, PR and HER2 status was routinely determined by pathologists using commercially based immunohistochemistry tests following removal, and was available from the medical record for 831 (99.2%), 831 (99.2%) and 829 (98.9%) of cases, respectively. Of all patients (n = 838), 176 (20.6%) were excluded because of lack of participation in HERPACC (n = 146), insufficient information on receptor status (n = 7), or a history of previous cancer (n = 23). Finally, 678 patients aged 19–79 years with a new histological diagnosis of breast cancer were considered eligible.

We randomly selected controls matched by age (±0 years) and menopausal status (premenopause or postmenopause) with a 1:5 case–control ratio from 9,343 women who were confirmed to be cancer-free by diagnostic procedure at our hospital and who had no prior history of cancer between 2001 and 2005. Eventually, 3,390 controls were included. Our previous study confirmed the feasibility of using noncancer outpatients at our hospital as controls in epide-

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miological studies because their general lifestyles are accordant with those of a general population randomly selected from the electoral roll in Nagoya City, Aichi Prefecture.[13] We assessed the clinical diagnosis among noncancer outpatients in the previous study and confirmed that 44% presented with no abnormal findings by examination, 35% had benign and nonspecific diseases (*e.g.*, mastitis: 7.5%, atrophic gastritis: 2.2%, myoma uteri: 1.7%, *etc.*), 13% had benign tumor and non-neoplastic polyp (*e.g.*, colonic polyp: 2.7%, *etc.*) and 3.4% had cystic disease (*e.g.*, breast cyst: 1.7%, *etc.*).[14]

Assessment of soybean intake and other exposure data

The FFQ consisted of 47 single food items with frequencies in the 8 categories of never or seldom, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, once/day, twice/day and 3+ times/day.[15–17] For staple foods such as rice, bread and noodles, the usual number of bowls or slices consumed on one time, as well as intake frequency, was inquired for breakfast, lunch and supper, separately. We asked the subjects about the average intake of frequency during the 1-year period preceding the onset of the present disease or before the interview. Dietary intake of soybean products was computed by multiplying the standard portion size of *tofu* (soybean curd), *miso* (fermented soybean paste) soup, *natto* (fermented soybeans), *aburage* (thinly sliced deep fried tofu) and frequency of consumption. The standard portion sizes in each soy food were calculated based on validity test using 3-day weighed dietary records. One serving in the soy foods was 50 g for *tofu*, 52 g for *miso* soup, 30 g for *natto* and 50 g in female for *aburage*. Similarly, total energy was computed by the standard portion size, frequency and energy (per gram) in foods as listed in the Standard Tables of Food Consumption and the Follow-up version.[18,19] Validity and reproducibility of the FFQ were acceptable.[16,17] The correlation coefficient for energy-adjusted intakes of soybeans was 0.53 in women. Energy-adjusted intake of soybean products was calculated by the residual method.[20]

Total alcohol consumption was estimated as the summed amount of pure alcohol consumption. Drinking habits were entered in the 4 categories of never, former, current moderate and heavy drinking. Heavy drinkers were defined as those currently drinking alcoholic beverages 5 days or more per week at a daily amount of 23 g (1 Japanese drink) or more, and moderate drinkers as those currently consuming less frequently than 5 days per week, in lower amounts, or both. Cumulative smoking dose was evaluated as pack-years, the product of the number of packs consumed per day and years of smoking. Smoking habit was entered under the 4 categories of never, former and current smoking of <20 and ≥ 20 pack-years. Former drinkers or smokers were defined as those who quit drinking or smoking at least 1 year before the survey, respectively.

Statistical analyses

To assess the strength of associations between the intake of soybean products or selected soy food items and risk of breast cancer, odd ratios (ORs) with 95% confidence intervals (CIs) were estimated using age- and menopausal status-matched conditional logistic models adjusted for potential confounders. Intake of soybean products was categorized into 3 groups as first (lowest), second, and third tertiles of dietary intake among controls. Intake frequencies of each soy food item were divided into 3 categories as first (lowest frequency group), second and third. Potential confounders considered in the multivariate analyses were age, drinking habit (never drinkers, former drinkers, moderate or heavy drinkers), smoking habit (never smokers, former smokers, current smokers of <20, or ≥ 20 pack-years), current body mass index (BMI) (<18.5, 18.5–24.9, ≥ 25.0), regular exercise (yes or no), family history of breast cancer (yes, no), total nonalcohol energy intake (as a continuous variable), multivitamin use (at least once per week for 1 year or longer: yes or no), menopausal status (premenopause, postmenopause), age at menarche (≤ 12, 13–14, ≥ 15), parity (0, 1–2, ≥ 3), past use of hormone-replacement therapy (never, 1–6 months, >6 months), referral pattern to our hospital (patient discretion, family or friend recommendation, referral from other clinics, secondary screening after primary screening or others) and age at menopause for postmenopausal women (≤ 47, 48–52, ≥ 53). We used noncancer patients at our hospital as controls, given the likelihood that our cases arose within this population base. To modify for any difference between cases and controls, we also adjusted for referral pattern. Differences in categorized demographic variables between the cases and controls were tested by the chi-square test. Mean values for total nonalcohol energy intake were compared for cases and controls by Student's t test. As a basis for the trend test, the median values of each tertile of soybean product consumption were included in the model, and we assigned the scores of 0, 1 and 2 to the first (lowest), second and third frequency group in the selected soy food items, respectively. A p value less than 0.05 was considered statistically significant. All analyses were performed using STATA version 10 (Stata Corp., College Station, TX).

Results

Data from 678 breast cancer cases and 3,390 controls were available for analysis. Table I shows the distribution of cases and controls by background characteristics according to menopausal status. Age and menopausal status were completely matched. In postmenopausal women, heavy drinkers were significantly more frequent among cases than controls ($p = 0.03$), as was the proportion of high BMI ($p < 0.01$). Compared with the controls, women with postmenopausal breast cancer were more likely to report a family history of breast cancer ($p < 0.01$). Among postmenopausal women, multivitamin supplementation was more prevalent in the controls ($p = 0.01$). With regard to referral pattern, family recommendation and referral from other clinics were more frequent among the case group, while patient discretion and secondary screening were less frequent in both premenopausal and postmenopausal women ($p < 0.01$).

Intake of soybean products was inversely associated with the overall risk of breast cancer (Table II). The OR was 0.80 (95% CI, 0.64–0.99) for the top tertile of soybean product intake compared with the lowest tertile of intake (trend $p = 0.03$). On analysis by menopausal status, the decreased risk was observed across menopausal status, though was not statistically significant. We therefore decided to examine risk for breast cancer combined in analysis by receptor status. On the other hand, analysis by type of soy foods for *miso* soup, *tofu*, *natto* and *aburage* did not show clearly association in overall and both premenopausal and postmenopausal women.

Of 678 breast cancer cases, cases positive for ER, PR and HER2 accounted for 536 (79.1%), 440 (64.9%) and 155 (22.9%) patients, respectively. When examined by joint ER/PR/HER2 status, 57 (8.4%) were ER+/PR+/HER2+, 378 (55.8%) were ER+/PR+/HER2−, 68 (10.0%) were ER−/PR−/HER2+, 69 (10.2%) were ER−/PR−/HER2− and 106 (15.6%) were other subtypes.

Table III shows the impact of soybean product consumption on breast cancer risk according to receptor status. Soybean product intake was associated with a significantly decreased risk of ER+ or HER2− breast cancer, with odds ratios in the top tertile of intake of 0.74 (95% CI, 0.58–0.94; trend $p = 0.01$) for ER+ tumors and 0.78 (95% CI, 0.61–0.99; trend $p = 0.04$) for HER2− tumors. The similar ORs were observed in PR+ and PR− tumor, although the results were not significant.

We further examined the impact of soybean product intake on breast cancer risk according to joint receptor status (Table IV). A significantly decreased risk of ER+/PR+/HER2− breast cancer with consumption of soybean products was observed (top tertile OR = 0.73, 95% CI: 0.54–0.97, trend $p = 0.03$). On analysis by menopausal status, a protective effect was found among premenopausal women (top tertile OR = 0.65, 95% CI: 0.43–0.96, trend $p = 0.03$). On the other hand, no association was found for other subtypes of breast cancer. In analysis according to receptor status, the association between the intake of soy food items and breast cancer risk was also unclear (data not shown).

TABLE I – CHARACTERISTICS OF CASES AND CONTROLS

	Premenopause			Postmenopause		
	Cases (n = 329) n (%)	Controls (n = 1,645) n (%)	P	Cases (n = 349) n (%)	Controls (n = 1,745) n (%)	P
Age						
18–29	10 (3.0)	50 (3.0)		0 (0)	0 (0)	
30–39	74 (22.5)	370 (22.5)		0 (0)	0 (0)	
40–49	176 (53.5)	880 (53.5)		14 (4.0)	70 (4.0)	
50–59	69 (21.0)	345 (21.0)		149 (42.7)	745 (42.7)	
60–69	0 (0)	0 (0)		141 (40.4)	705 (40.4)	
70–79	0 (0)	0 (0)	1.00	45 (12.9)	225 (12.9)	1.00
Drinking habit						
Never	188 (57.1)	899 (54.7)		228 (65.3)	1,175 (67.3)	
Former[1]	4 (1.2)	31 (1.9)		3 (0.9)	32 (1.8)	
Current						
Moderate[2]	112 (34.0)	598 (36.4)		90 (25.8)	454 (26.0)	
Heavy[3]	23 (7.0)	97 (5.9)	0.59	24 (6.9)	63 (3.6)	0.03
Unknown	2 (0.6)	20 (1.2)		4 (1.1)	21 (1.2)	
Smoking habit						
Never	254 (77.2)	1,233 (75.0)		308 (88.3)	1,486 (85.2)	
Former[1]	17 (5.2)	96 (5.8)		10 (2.9)	77 (4.4)	
Current (pack years)						
0–19	41 (12.5)	229 (13.9)		12 (3.4)	79 (4.5)	
≥20	15 (4.6)	81 (4.9)	0.83	14 (4.0)	92 (5.3)	0.30
Unknown	2 (0.6)	6 (0.4)		5 (1.4)	11 (0.6)	
BMI						
<18.5	40 (12.2)	178 (10.8)		17 (4.9)	125 (7.2)	
18.5–24.9	250 (76.0)	1,257 (76.4)		225 (64.5)	1,280 (73.4)	
≥25.0	35 (10.6)	199 (12.1)	0.63	106 (30.4)	323 (18.5)	<0.01
Unknown	4 (1.2)	11 (0.7)		1 (0.3)	17 (1.0)	
Regular exercise						
Yes	226 (68.7)	1,132 (68.8)		252 (72.2)	1,321 (75.7)	
No	103 (31.3)	511 (31.1)	0.94	90 (25.8)	413 (23.7)	0.32
Unknown	0 (0)	2 (0.1)		7 (2.0)	11 (0.6)	
Family history of breast cancer						
Yes	17 (5.2)	102 (6.2)		38 (10.9)	106 (6.1)	
No	290 (88.1)	1,396 (84.9)	0.41	282 (80.8)	1,476 (84.6)	<0.01
Unknown	22 (6.7)	147 (8.9)		29 (8.3)	163 (9.3)	
Age at menarche						
≤12	154 (46.8)	685 (41.6)		68 (19.5)	316 (18.1)	
13–14	140 (42.6)	777 (47.2)		182 (52.1)	808 (46.3)	
≥15	33 (10.0)	176 (10.7)	0.21	94 (26.9)	562 (32.2)	0.09
Unknown	2 (0.6)	7 (0.4)		5 (1.4)	59 (3.4)	
Age at menopause						
≤47				84 (24.1)	395 (22.6)	
48–52				164 (47.0)	909 (52.1)	
≥53				93 (26.6)	418 (24.0)	0.27
Unknown				8 (2.3)	23 (1.3)	
Parity						
0	68 (20.7)	325 (19.8)		28 (8.0)	165 (9.5)	
1–2	197 (59.9)	994 (60.4)		240 (68.8)	1,105 (63.3)	
≥3	64 (19.5)	321 (19.5)	0.94	81 (23.2)	464 (26.6)	0.20
Unknown	0 (0)	5 (0.3)		0 (0)	11 (0.6)	
Hormone replacement therapy (months)						
Never	288 (87.5)	1,377 (83.7)		286 (81.9)	1,428 (81.8)	
1–6	25 (7.6)	151 (9.2)		32 (9.2)	151 (8.7)	
>6	13 (4.0)	87 (5.3)	0.34	22 (6.3)	121 (6.9)	0.88
Unknown	3 (0.9)	30 (1.8)		9 (2.6)	45 (2.6)	
Mean total nonalcohol energy, kcal/day (SD)	1,470.0 (262.3)	1,490.1 (284.7)	0.24	1,508.0 (277.9)	1,497.7 (273.4)	0.52
Multivitamin use (at least once per week for 1 year or longer)						
Yes	66 (20.1)	346 (21.0)		65 (18.6)	430 (24.6)	
No	254 (77.2)	1,257 (76.4)	0.70	270 (77.4)	1,234 (70.7)	0.01
Unknown	9 (2.7)	42 (2.6)		14 (4.0)	81 (4.6)	
Referral pattern to our hospital						
Patient's discretion	88 (26.7)	482 (29.3)		106 (30.4)	647 (37.1)	
Family recommendation	77 (23.4)	304 (18.5)		68 (19.5)	255 (14.6)	
Referral from other clinics	92 (28.0)	305 (18.5)		101 (28.9)	357 (20.5)	
Secondary screening after primary screening	65 (19.8)	541 (32.9)		69 (19.8)	460 (26.4)	
Others	3 (0.9)	9 (0.5)	<0.01	1 (0.3)	9 (0.5)	<0.01
Unknown	4 (1.2)	4 (0.2)		4 (1.1)	17 (1.0)	

SD, standard deviation; BMI, body mass index.
[1]Former smokers and drinkers were defined as subjects who had quit smoking and drinking at least 1 year previously.–[2]Moderate drinker means less 23 g ethanol/drink and/or less 5 days/week.–[3]Heavy drinker means 23 g ethanol/drink or more and 5 days/week or more.

TABLE II – ADJUSTED ODDS RATIOS (OR) AND 95% CONFIDENCE INTERVALS (CI) FOR THE ASSOCIATION BETWEEN SOYBEAN PRODUCTS AND SOY FOODS INTAKE AND BREAST CANCER RISK ACCORDING TO MENOPAUSAL STATUS

	All		Premenopause		Postmenopause	
	Cases/controls (n = 678/3,390)	ORs[1] (95% CI)	Cases/controls (n = 329/1,645)	ORs[1] (95% CI)	Cases/controls (n = 349/1,745)	ORs[1] (95% CI)
Soybean products (g/day)						
Tertile 1 (1.1–27.4)	242/1,108	1.00 (Referent)	145/641	1.00 (Referent)	97/467	1.00 (Referent)
Tertile 2 (27.4–51.2)	235/1,108	0.95 (0.77, 1.16)	106/530	0.85 (0.64, 1.13)	129/578	1.01 (0.75, 1.39)
Tertile 3 (51.2–326.3)	195/1,108	0.80 (0.64, 0.99)	74/442	0.74 (0.54, 1.02)	121/666	0.84 (0.61, 1.15)
Unknown	6/66		4/32		2/34	
p_{trend}		0.03		0.06		0.17
Miso soup						
≤2 times/week	200/973	1.00 (Referent)	113/516	1.00 (Referent)	87/457	1.00 (Referent)
3–6 times/week	255/1,287	0.97 (0.79, 1.20)	132/696	0.89 (0.67, 1.18)	123/591	1.07 (0.78, 1.47)
≥1 time/day	216/1,078	0.99 (0.79, 1.24)	81/418	0.91 (0.66, 1.27)	135/660	1.08 (0.79, 1.48)
Unknown	7/52		3/15		4/37	
p_{trend}		0.93		0.58		0.65
Tofu						
≤3 times/month	209/1,006	1.00 (Referent)	120/556	1.00 (Referent)	89/450	1.00 (Referent)
1–2 times/week	259/1,286	0.98 (0.80, 1.21)	126/629	0.95 (0.71, 1.27)	133/657	1.00 (0.73, 1.37)
≥3 times/week	193/1,027	0.89 (0.72, 1.12)	78/441	0.84 (0.61, 1.17)	115/586	0.93 (0.68, 1.28)
Unknown	17/71		5/19		12/52	
p_{trend}		0.30		0.32		0.56
Natto						
≤3 times/month	197/912	1.00 (Referent)	114/516	1.00 (Referent)	83/396	1.00 (Referent)
1–2 times/week	241/1,160	0.96 (0.78, 1.19)	131/593	1.04 (0.78, 1.39)	110/567	0.91 (0.66, 1.27)
≥3 times/week	230/1,259	0.87 (0.70, 1.08)	81/517	0.72 (0.52, 0.99)	149/742	0.99 (0.72, 1.36)
Unknown	10/59		3/19		7/40	
p_{trend}		0.20		0.06		0.95
Aburage						
Seldom	57/331	1.00 (Referent)	41/206	1.00 (Referent)	16/125	1.00 (Referent)
1–3 times/month	285/1,336	1.21 (0.88, 1.66)	150/729	1.10 (0.73, 1.65)	135/607	1.59 (0.90, 2.81)
≥1 time/week	329/1,667	1.11 (0.80, 1.54)	135/689	1.06 (0.70, 1.61)	194/978	1.37 (0.78, 2.41)
Unknown						
p_{trend}	7/56	0.99	3/21	0.93	4/35	0.97

[1]Conditional logistic regression model additionally controlling for drinking habit, smoking habit, BMI, regular exercise, family history of breast cancer, total nonalcohol energy intake, multivitamin use, age at menarche, parity, hormone-replacement therapy, referral pattern to our hospital and age at menopause for postmenopausal women.

Discussion

Our case–control study in a population derived from hospital outpatients with adjustment for various lifestyle factors suggested that a high intake of soybean products was associated with a decreased risk of ER+, HER2− and ER+/PR+/HER2− breast cancer. These findings indicate that the protective effect of soy against breast cancer risk differs by receptor status.

Ecologic studies have shown that breast cancer incidence is lower in populations with habitually high soy food consumption.[21] Various vegetables and grains contain small amounts of isoflavones, but far higher quantities are found in soybeans, and accordingly the impact of soy food intake on breast cancer risk has been extensively investigated. Results from epidemiologic studies of this association have varied. One prospective[22] and 3 case–control studies[23–25] showed significant associations between soy food or isoflavone intake and the risk of breast cancer overall; 2 showed protective associations in premenopausal women only[26–28]; while other cohort[29–31] and case–control studies[32–38] showed no association. Recent meta-analysis have reported that soy intake was associated with a small reduction in breast cancer risk[3]; however, one of the problems in conducting the meta-analysis was that the measures used to quantify soy intake varied considerably across studies.

This inconsistency in these studies may in part be due to their lack of differentiation of receptor status in breast cancer tissue, as well as to errors in soybean intake assessment and confounding. Several studies of representative risk factors have focused on determining the etiologies of breast cancer tumors classified by joint ER, PR and HER2 status. Results suggested substantial heterogeneity in causation, and tumors subclassified by receptor status may actually represent distinct forms of breast cancer with differing etiologies. Previous studies have reported that hormonal factors, including age at menarche,[5] parity,[7] age at first pregnancy[6,7] and BMI[39] may be more strongly associated with an increased risk of ER+ and/or PR+ than of ER− and/or PR− breast cancer. In present study, age at menarche was associated with ER+ and PR+ breast cancers (data not shown). To date, however, only one study has examined the association between soy intake and breast cancer subtype defined by receptor status; that study, conducted in China, reported that risk reduction with soy protein intake was stronger for breast cancer positive for ER+/PR+ than for other ER/PR status.[34] To our knowledge, the present study is the first to include HER2 status.

Endogenous estrogen has been clearly recognized as a cause of breast cancer, and hormonal therapy with estrogen for menopause is associated with an increased risk of breast cancer.[40] Isoflavones have been suggested to reduce circulating estrogen levels, but the hypothesis has not been confirmed.[41] A more plausible explanation for the protective effect of soy intake on breast cancer risk may be that since Isoflavones bind preferentially to activate ER-β,[42,43] although they can bind to both ER-α and ER-β, and ER-β might inhibit the activation of ER-α.[44] Given the anticarcinogenic properties of soybeans, our finding that the protective effect of soy intake was more pronounced in ER-positive breast cancer may be plausible. Interestingly, a protective effect was seen only in HER2− breast cancer with ER+/PR+, not in HER2+ or ER+/PR+ cases. Clinical studies have demonstrated that overexpression of HER2 occurs in 20% of breast tumors and has been associated with a poor prognosis compared with HER2− breast cancer. Although the mechanism by which HER2 is selectively overexpressed in cancers remains poorly understood, the absence of expression of hormone receptors in many HER2+ tumors and unresponsiveness to tamoxifen suggests that positivity is associated with hormone independence.[45,46] In a previous epidemiological study, parity and age at first pregnancy were associated with

TABLE III – ADJUSTED ODDS RATIOS (OR) AND 95% CONFIDENCE INTERVALS (CI) FOR THE ASSOCIATION BETWEEN SOYBEAN PRODUCTS INTAKE AND BREAST CANCER RISK ACCORDING TO RECEPTOR STATUS

Soybean products (g/day)	ER+		ER−		PR+		PR−		HER2+		HER2−	
	Cases/controls (n = 536/2,680)	ORs[1] (95% CI)	Cases/controls (n = 142/710)	ORs[1] (95% CI)	Cases/controls (n = 440/2,200)	ORs[1] (95% CI)	Cases/controls (n = 238/1,190)	ORs[1] (95% CI)	Cases/controls (n = 155/775)	ORs[1] (95% CI)	Cases/controls (n = 523/2,615)	ORs[1] (95% CI)
Tertile 1 (1.1–27.4)	195/877	1.00 (Referent)	47/231	1.00 (Referent)	157/719	1.00 (Referent)	85/389	1.00 (Referent)	53/272	1.00 (Referent)	189/836	1.00 (Referent)
Tertile 2 (27.4–51.2)	189/865	0.96 (0.76, 1.21)	46/243	0.84 (0.52, 1.34)	156/720	0.98 (0.76, 1.27)	79/388	0.86 (0.61, 1.23)	62/260	1.15 (0.75, 1.76)	173/848	0.88 (0.69, 1.12)
Tertile 3 (51.2–326.3)	147/883	0.74 (0.58, 0.94)	48/225	0.95 (0.58, 1.57)	124/715	0.78 (0.60, 1.03)	71/393	0.79 (0.55, 1.16)	37/235	0.82 (0.50, 1.33)	158/873	0.78 (0.61, 0.99)
Unknown	5/55		1/11		3/46		3/20		3/8		3/58	
p_{trend}		0.01		0.94		0.05		0.24		0.33		0.04

TABLE IV – ADJUSTED ODDS RATIOS (OR) AND 95% CONFIDENCE INTERVALS (CI) FOR THE ASSOCIATION BETWEEN SOYBEAN PRODUCTS INTAKE AND BREAST CANCER RISK ACCORDING TO 3 RECEPTOR STATUS

Soybean products (g/day)	ER+/PR+/HER2+		ER+/PR+/HER2−		ER−/PR−/HER2+		ER−/PR−/HER2−	
	Cases/controls (n = 57/285)	ORs[1] (95% CI)	Cases/controls (n = 378/1,890)	ORs[1] (95% CI)	Cases/controls (n = 68/340)	ORs[1] (95% CI)	Cases/controls (n = 69/345)	ORs[1] (95% CI)
Tertile 1 (1.1–27.4)	17/104	1.00 (Referent)	139/606	1.00 (Referent)	20/112	1.00 (Referent)	26/110	1.00 (Referent)
Tertile 2 (27.4–51.2)	24/101	1.46 (0.63, 3.40)	129/611	0.91 (0.69, 1.21)	27/116	1.19 (0.58, 2.41)	16/119	0.43 (0.20, 0.92)
Tertile 3 (51.2–326.3)	15/77	1.28 (0.51, 3.27)	108/630	0.73 (0.54, 0.97)	20/109	1.12 (0.52, 2.44)	27/108	0.93 (0.44, 1.98)
Unknown	1/3		2/43		1/3		0/8	
p_{trend}		0.83		0.03		0.92		0.90

[1]Conditional logistic regression model additionally controlling for drinking habit, smoking habit, BMI, regular exercise, family history of breast cancer, total nonalcohol energy intake, multivitamin use, age at menarche, parity, hormone-replacement therapy and referral pattern to our hospital.

HER2− breast cancer risk, but not with HER2+ risk,[7] suggesting that hormonal factors influence HER2− breast cancer only, whereas HER2+ tumors develop uninfluenced by these factors even if both ER and PR are positive. Our finding of a decreased risk with soybean intake only in HER2− and ER+/PR+ cases appears compatible with the consideration that soy affects breast cancer risk mainly *via* its antiestrogenic effect.

In Japan, the main sources of soybean intake are *tofu*, *miso* soup and *natto*. In our previous study, *tofu* was protective for premenopausal breast cancer.[28] In contrast, a second Japanese study reported an inverse association with *miso* soup consumption,[22] while a third found no association with breast cancer risk for any soy food.[31] In our analysis of frequency of soy food intake (times/month, week or day), we did not observe clear association with intake of specific soy foods, but did see an association with the amount of soybean intake (g/day). These results suggest that estimation based on a validated food frequency questionnaire may be more sensitive than that by the frequency of specific food items. Further investigation of this point is warranted.

Our study has several methodological strengths. First, age and menopausal status confounding could be completely controlled by exact matching of these factors. The matched design validates a better estimate of menopausal status-based analysis. Second, since complete receptor status was known for nearly all cases, selection bias in the cases was negligible. Third, soybean intake was estimated using a validated questionnaire. In addition, among Japanese, *tofu*, *miso* soup and *natto* contributed more than 80% of the total genistein intake, one of several known isoflavones[28]; thus, soy foods in this study is likely to cover soybean products in Japan.

Several methodological limitations warrant consideration. First, as with other hospital-based case–control studies, the controls may have differed from the general population. Our previous comparison of lifestyle characteristics of HERPACC controls and individuals selected randomly from the general population in Nagoya City, however, confirmed no substantial difference.[13] Like most general hospitals in Japan, our hospital accepts new outpatients who visit of their own volition, with or without a doctor's referral, notwithstanding our description as a "Cancer Center." Second, although we used a self-administered questionnaire to evaluate soybean product intake, data obtained from an FFQ may not accurately reflect intake. If present, however, any such misclassification would be nondifferential, and would likely underestimate the causal association. Third, as with other case–control studies, we are completely unable to ignore recall of diet. Although the questionnaires were completed prior to the examination in our hospital, some case patients referred to the hospital might have known the diagnosis. It is unlikely, however, that the recall bias affected the findings differentially between receptor positive and negative breast cancers. Forth, we cannot exclude the possibility of residual confounding by other dietary characteristics. Finally, the limited number of rare subtype in breast cancer cases indicates the need for replication of our findings in a larger study.

In conclusion, our study shows that the intake of soybean products significantly reduces the risk of ER+/PR+/HER2− breast cancer. These findings are biologically plausible, and suggest a potential beneficial effect of soybean products in the prevention of breast cancer.

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Short Communication

Alcohol Drinking and One-Carbon Metabolism-Related Gene Polymorphisms on Pancreatic Cancer Risk

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Abstract

Effect of alcohol consumption on pancreatic cancer risk has been investigated in many studies, but results have been inconsistent. We conducted a case-control study to assess the effect of alcohol on pancreatic cancer in conjunction with polymorphisms in one-carbon metabolism enzymes, methylenetetrahydrofolate reductase (*MTHFR* C677T), methionine synthase (*MTR* A2756G), methionine synthase reductase (*MTRR* A66G), and thymidylate synthase (*TS*) variable number of tandem repeat. A total of 157 pancreatic cancer patients and 785 age- and sex- matched control subjects were genotyped for polymorphisms. Odds ratios (OR) with 95% confidence intervals (95% CI) were estimated using unconditional logistic models adjusted for potential confounders. Heavy alcohol drinking was marginally associated with an increased risk of pancreatic cancer (OR, 1.90; 95% CI, 1.00-3.62). None of the polymorphisms showed any significant effect on pancreatic cancer risk by genotype alone. In stratified analysis, effect of alcohol consumption on pancreatic cancer was observed in individuals with the *MTHFR* 667 CC, *MTR* 2756 AA, or *MTRR* 66 G allele. OR (95% CI) of pancreatic cancer for heavy drinkers compared with never drinkers was 4.50 (1.44-14.05) in the *MTHFR* 667 CC genotype, 2.65 (1.17-6.00) in the *MTR* 2756 AA genotype, and 3.35 (1.34-8.36) in the *MTRR* 66 G allele carriers. These results suggest that the folate-related enzyme polymorphism modifies the association between drinking habit and pancreatic cancer risk. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2742–7)

Introduction

A high intake of folate, which is plentiful in vegetables and fruits, has been associated with a reduced risk of several cancers (1). Folate functions within so-called "one-carbon metabolism" to facilitate *de novo* deoxynucleoside triphosphate synthesis and to provide the methyl groups required for intracellular methylation reactions. Epidemiologic studies have suggested the importance of folate in pancreatic cancer risk (2, 3). Polymorphisms in critical enzymes involved in the one-carbon metabolism pathway, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthase (TS), play important and interrelated roles in folate metabolism and may thereby influence the risk of pancreatic cancer.

Heavy alcohol consumption is known to be a major cause of chronic pancreatitis, and chronic pancreatitis has been linked to pancreatic cancer (4); however, the association between alcohol consumption and risk of pancreatic cancer has been inconsistent (5, 6). Chronic inflammation in pancreatitis induces DNA damage and mutations and thereby facilitates the development of pancreatic cancer. DNA synthesis for replication and repair is largely dependent on the availability of the one-carbon metabolism pathway. Therefore, the one-carbon metabolism polymorphisms may modify influence of alcohol drinking on pancreatic cancer risk.

Here, we evaluated the effect of alcohol consumption in conjunction with genetic polymorphisms in one-carbon metabolism enzymes on pancreatic cancer risk among Japanese.

Materials and Methods

Study Population. The subjects, ages 20 to 79 years, in the present study were enrolled between January 2001 and November 2005 in the framework of Hospital-based Epidemiologic Research Program at Aichi Cancer Center (7, 8). In brief, Hospital-based Epidemiologic Research Program at Aichi Cancer Center -II was launched in 2001, asking all first-visit outpatients in Aichi Cancer Center Hospital to provide 7 mL blood as well as information on lifestyle factors. A total of 35,838 patients visited Aichi

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Cancer Center Hospital as first-visit outpatients during this period. Among them, 6,300 patients were not enrolled due to miscellaneous reasons; 28,571 (79.7%) completed the questionnaire adequately. Of those who completed an interview, 50.7% donated a blood sample. All participants gave written informed consent and the study was approved by the Ethics Committee of Aichi Cancer Center.

A total of 175 patients who were newly diagnosed as having pancreatic cancer (*International Classification of Disease, Tenth Edition* code C25) at our hospital were deemed to be potential cases. From these, we excluded 16 patients with a past history of cancer and 2 patients with pancreatic endocrine tumor, leaving 157 cases eligible for analysis. Control subjects were randomly selected from first-visit outpatients. A total of 7,240 individuals who were confirmed not to have cancer according to the cancer registry and medical record was deemed to be potential controls. We excluded 276 patients with a past history of cancer, leaving 6,964 controls eligible for analysis. Eventually, 785 controls were individually matched with case subjects by age (±3 years) and sex at a 1:5 case-control ratio. We assessed the clinical diagnosis among noncancer outpatients in the previous study and confirmed that a large fraction of the patients had no abnormal findings by examination and nonspecific diseases (9). All subjects for the present study were Japanese and most subjects are living in and around Aichi Prefecture, central Japan.

Genotyping of MTHFR, MTR, MTRR, and TS. Genotyping for *MTHFR* C677T (dbSNP ID: rs1801133), *MTR* A2756G (rs1805087), and *MTRR* A66G (rs1801394) was based on TaqMan Assays by Applied Biosystems. The *TS* variable number of tandem repeat polymorphism was defined by PCR using 5′-CGTGGCTCCTGCGTTTCC-3′ and 5′-GAGCCGGCCACAGGCAT-3′ primers.

Assessment of Exposures. Daily alcohol consumption in grams was determined by summing the pure alcohol amount in the average daily consumption of Japanese

Table 1. Characteristics of case and control subjects

	Cases (n = 157), n (%)	Controls (n = 785), n (%)	P
Age (y)			
20-39	8 (5.1)	43 (5.5)	
40-49	15 (9.6)	66 (8.4)	
50-59	52 (33.1)	241 (30.7)	
60-69	55 (35.0)	292 (37.2)	
70-79	27 (17.2)	143 (18.2)	0.948
Sex			
Male	112 (71.3)	560 (71.3)	
Female	45 (28.7)	225 (28.7)	1.000
Drinking habit			
Never	46 (29.3)	306 (39.0)	
Former	10 (6.4)	31 (3.9)	
Current			
Moderate*	73 (46.5)	347 (44.2)	
Heavy†	25 (15.9)	88 (11.2)	0.054
Unknown	3 (1.9)	13 (1.7)	
Smoking habit			
Never	53 (33.8)	325 (41.4)	
Former	43 (27.4)	218 (27.8)	
Current (pack-years)			
0-39	23 (14.6)	135 (17.2)	
≥40	37 (23.6)	102 (13.0)	0.006
Unknown	1 (0.6)	5 (0.6)	
Body mass index			
<18.5	13 (8.3)	39 (5.0)	
18.5-24.9	110 (70.1)	544 (69.3)	
≥25.0	34 (21.7)	195 (24.8)	0.209
Unknown	0 (0)	7 (0.9)	
Mean (SD) total nonalcohol energy, kcal/d	1,599.8 (367.3)	1,601.6 (348.3)	0.955
Folate intake (μg/d)			
Tertile 1 (148.6-274.3)	54 (34.4)	260 (33.1)	
Tertile 2 (274.5-360.5)	62 (39.5)	259 (33.0)	
Tertile 3 (360.9-980.7)	40 (25.5)	259 (33.0)	0.135
Unknown	1 (0.6)	7 (0.9)	
History of diabetes mellitus			
Yes	34 (21.7)	67 (8.5)	
No	123 (78.3)	718 (91.5)	<0.001
Referral pattern to our hospital			
Patient discretion	19 (12.1)	247 (31.5)	
Family recommendation	22 (14.0)	146 (18.6)	
Referral from another clinic	92 (58.6)	193 (24.6)	
Secondary screening after primary screening	22 (14.0)	186 (23.7)	
Other	0 (0)	7 (0.9)	<0.001
Unknown	2 (1.3)	6 (0.8)	

*Moderate drinker means <46 g ethanol/d or <5 d/wk.
†Heavy drinker means ≥46 g ethanol/d on ≥5 d/wk.

Table 2. Effect of one-carbon metabolism-related polymorphisms on pancreatic cancer risk

	No. cases/controls	OR* (95% CI)	P_{trend}
MTHFR (C677T)			
CC	57/291	1.00 (reference)	
CT	80/366	0.98 (0.65-1.47)	
TT	20/128	0.75 (0.41-1.35)	0.415
CT + TT	100/494	0.92 (0.63-1.36)	0.687
MTR (A2756G)			
AA	104/524	1.00 (reference)	
AG	47/236	0.91 (0.61-1.38)	
GG	6/23	1.39 (0.50-3.88)	0.856
Unknown	0/2		
AG + GG	53/259	0.95 (0.64-1.42)	0.809
MTRR (A66G)			
AA	78/374	1.00 (reference)	
AG	67/330	0.88 (0.59-1.30)	
GG	12/81	0.78 (0.39-1.56)	0.388
AG + GG	79/411	0.86 (0.59-1.25)	0.424
TS			
Non-2R/non-2R	101/548	1.00 (reference)	
2R/non-2R	51/217	1.41 (0.94-2.11)	
2R/2R	5/20	1.48 (0.49-4.47)	0.095
2R/non-2R + 2R/2R	56/237	1.41 (0.95-2.09)	0.085

*Adjusted for age, sex, drinking habit, smoking habit, body mass index, total nonalcohol energy intake, dietary folate intake, history of diabetes mellitus, and referral pattern to our hospital.

sake (rice wine), shochu (distilled spirit), beer, wine, and whiskey, with one cup of Japanese sake (180 mL) considered equivalent to 23 g ethanol, one drink of shochu (180 mL) to 46 g, one large bottle of beer (720 mL) to 23 g, one glass of wine (80 mL) to 9.2 g, and one shot of whiskey (28.5 mL) to 11.5 g. Heavy drinkers were defined as those currently drinking alcoholic beverages ≥5 days/wk in a daily amount of ≥46 g (two Japanese drinks), whereas moderate drinkers were defined as those currently consuming less frequently than 5 days/wk, in lower amounts, or both. Former drinkers or smokers were defined as those who quit drinking or smoking at least 1 year before the survey, respectively. Dietary intake of folate was computed based on the food frequency questionnaire consisted of 47 single food items (10). The deattenuated correlation coefficients for energy-adjusted intakes of folate using 3-day weighed dietary records were 0.36 [95% confidence interval (95% CI), 0.12-0.58] in men and 0.38 (95% CI, 0.25-0.62) in women (11).

Statistical Analysis. To assess the strength of the associations between alcohol consumption, polymorphisms of folate metabolism enzyme, and pancreatic cancer risk, odds ratios (OR) with 95% CI were estimated using unconditional logistic models adjusted for potential confounders. Potential confounders considered in the multivariate analyses were age, sex, drinking habit (never, former, moderate, or heavy drinkers), smoking habit (never, former, or current smokers of <40 or ≥40 pack-years), current body mass index (<18.5, 18.5-24.9, or ≥25.0), total nonalcohol energy intake (as a continuous variable), dietary folate intake (μg/d, tertiles), history of diabetes mellitus (yes or no), and referral pattern to our hospital (patient discretion, family or friend recommendation, referral from another clinic, secondary screening after primary screening, or others). Accordance with the Hardy-Weinberg equilibrium was checked for controls using the χ^2 test and used to assess any discrepancies between genotype and allele frequencies. To exclude the subjects who stop drinking due to pancreatic cancer, analysis without former drinkers was conducted for association with alcohol drinking. Interactions were assessed by the logistic model, which included interaction terms between alcohol consumption and genes with scores of genotype and drinking habit. $P < 0.05$ was considered statistically significant. Analyses of the risk estimate were performed using STATA version 10 (Stata).

Results

Table 1 shows the distribution of cases and controls by background characteristics. The proportion of current smokers of ≥40 pack-years was significantly higher in cases than controls ($P = 0.006$). A significantly high frequency of a history of diabetes mellitus was seen in cases ($P < 0.001$).

Genotype frequencies for all polymorphisms were in accordance with the Hardy-Weinberg law in controls (Table 2). None of the polymorphisms showed any significant effect on pancreatic cancer risk by genotype.

Heavy alcohol drinking was marginally associated with an increased risk of pancreatic cancer in overall analysis (OR, 1.90; 95% CI, 1.00-3.62; $P_{trend} = 0.036$; Table 3). To assess the effect of alcohol consumption and the one-carbon metabolism-related gene polymorphisms in pancreatic cancer risk, furthermore, we conducted the stratified analysis by the genotypes. Among subjects with the *MTHFR* 677 CC genotype, adjusted OR (95% CI) of pancreatic cancer was 4.50 (1.44-14.05) for heavy drinkers relative to never drinker ($P_{trend} = 0.008$). In contrast, the trend was not significant among those with *MTHFR* 677 CT or TT genotype. Heavy drinkers with *MTR* AA genotype or *MTRR* 66 G allele had higher risk of pancreatic cancer relative to never drinkers with these genotypes, whereas no association was observed in other genotypes. We examined the association between alcohol consumption and pancreatic cancer risk by folate intake; no clear interaction was found (data not shown).