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Soy Product and Isoflavone Consumption in Relation to Prostate Cancer in Japanese Men

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Abstract

The incidence of prostate cancer is much lower in Asian than Western populations. Environmental factors, such as dietary habits, may play a major role in the causation of prostate cancer. Although isoflavones have been suggested to show a preventive effect against prostate cancer in animal experiments, the results of epidemiologic studies are inconsistent. Here, we conducted a population-based prospective study in 43,509 Japanese men ages 45 to 74 years who generally have a high intake of isoflavones and low incidence of prostate cancer. Participants responded to a validated questionnaire, which included 147 food items. During follow-up from 1995 through 2004, 307 men were newly diagnosed with prostate cancer, of which 74 cases were advanced, 220 cases were organ localized, and 13 cases were of an undetermined stage. Intakes of genistein, daidzein, miso soup, and soy food were not associated with total prostate cancer. However, these four items decreased the risk of localized prostate cancer. In contrast, positive associations were seen between isoflavones and advanced prostate cancer. These results were strengthened when analysis was confined to men ages >60 years, in whom isoflavones and soy food were associated with a dose-dependent decrease in the risk of localized cancer, with relative risks for men in the highest quartile of genistein, daidzein, and soy food consumption compared with the lowest of 0.52 [95% confidence interval (95% CI), 0.30-0.90], 0.50 (95% CI, 0.28-0.88), and 0.52 (95% CI, 0.29-0.90), respectively. In conclusion, we found that isoflavone intake was associated with a decreased risk of localized prostate cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(3):538–45)

Introduction

The incidence of prostate cancer is much lower in Asian than in Western populations (1). However, Japanese migrants to the United States and Brazil have an increased incidence (2, 3), and the incidence of latent or clinically insignificant prostate cancer in autopsy studies among men from Asian countries and the United States is similar (4, 5). It has therefore been suggested that environmental factors may play an important role in the progression of prostate cancer. Asian populations consume large quantities of soy food that contained isoflavones such as genistein and daidzein (6). Mean serum or plasma concentrations of isoflavones in Japanese men are 10 to 100 times higher than those in men from the United Kingdom (7) and Finland (8). Moreover, Morton et al. (9) reported a higher concentration of daidzein in the prostatic fluid of Asian men than in Western men. Genistein and daidzein exhibit anticarcinogenic properties and estrogenic activity *in vitro* and have shown a protective effect against prostate cancer development in some animal studies (6). On these bases, isoflavones have been recognized as key substances that may decrease the incidence of prostate cancer in Asia. However, previous findings from epidemiologic studies regarding isoflavone or soy food intake and prostate cancer are equivocal (10-17).

This inconsistency may be due to errors in exposure measurement and limited variation in soy intake. Some of the previous epidemiologic studies investigated association between prostate cancer and a single soy food only, such as tofu or soy milk, and most were conducted in Western countries, in which physiologically meaningful amounts of soy are not consumed (10-12). Here, we investigated the association between isoflavone intake and risk of prostate cancer in a prospective study in Japanese who consume large amounts of soy.

Materials and Methods

Study Population. The Japan Public Health Center–Based Prospective Study was initiated in 1990 for cohort I and in 1993 for cohort II. The study design has been described in detail previously (18). Cohort I included those residents ages 40 to 59 years who had registered their addresses in five public health center areas (Iwate, Akita, Nagano, Okinawa, and Tokyo). Cohort II included those residents ages 40 to 69 years who had registered in six public health center areas (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka). The Tokyo subjects were not included in the data analysis because incidence data were not available. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan. The initial cohort consisted of 68,557 men.

Food Frequency Questionnaire. At baseline, participants completed a self-administered questionnaire that assessed information on lifestyle factors, medical, and smoking histories. The food frequency questionnaire (FFQ) in the baseline survey had 44 food items for cohort I and 52 food items for cohort II with four (cohort I) or five (cohort II) frequency categories but without standard portions/units. In contrast, the 5-year follow-up survey included a self-administered FFQ, which included lifestyle factors, medical history, and 147 food

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Study Group members are listed in Appendix A.

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and beverage items with standard portions/units and nine frequency categories. Owing to this greater detail, the present study therefore used the 5-year follow-up survey as baseline and followed the subjects from 1995 for cohort I and from 1998 for cohort II until 2004. After the 5-year follow-up survey, 128 subjects were found to be ineligible and were excluded because of non-Japanese nationality (n = 28), late report of emigration occurring before the start of the follow-up period (n = 97), incorrect birth data (n = 3), and subjects with self-reported prostate cancer (n = 21), leaving 58,427 men eligible for participation. Among eligible subjects, 46,001 men (79%) returned valid responses to the 5-year follow-up FFQ.

We dealt with two item groups: consumption of miso soup and soy food. Soy food referred to the consumption of "Tofu, Yushidofu (pre-drained tofu), Koyadofu (freeze-dried tofu), Aburaage (deep-fried tofu), Natto (fermented soybean), and soymilk," for which the major ingredient is soybeans. The questionnaire asked about the usual consumption of 147 foods and beverages during the previous year. The frequency of miso soup consumption was divided into six categories (almost never, 1-3 days/mo, 1-2 days/wk, 3-4 days/wk, 5-6 days/wk, and daily). Portion sizes were specified, and the amounts provided in three categories (less than half, same, and more than 1.5 times). One bowl of miso soup was calculated as 150 mL. Nine frequency categories were used for soy foods (almost never, 1-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, once a day, 2-3 times per day, 4-6 times per day, and ≥7 times per day). Portion sizes were specified, and the amounts were determined in three categories (less than half, same, and more than 1.5 times). Ten frequency categories were used for soy milk (almost never, 1-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, 1 glass per day, 2-3 glasses per day, 4-6 glasses per day, 7-9 glasses per day, and >9 glasses per day). The total consumption of miso soup (mL/d) and soy food (g/d) was calculated from these responses, whereas that of isoflavones (daidzein and genistein) was calculated using values in a specially developed food composition table for isoflavones in Japanese foods (19, 20).

Validity was assessed among subsamples using 14- or 28-day dietary records. Spearman's correlation coefficients between the energy-adjusted intake of miso soup and soy food consumption from the questionnaire and from dietary records were 0.54 and 0.53 for cohort I and 0.48 and 0.52 for cohort II, respectively, whereas those for energy-adjusted intake of daidzein and genistein were 0.65 and 0.65 for cohort I and 0.49 and 0.48 for cohort II, respectively. Moreover, Spearman's correlation coefficients for daidzein and genistein between energy-adjusted intakes from FFQ and those from serum concentration were 0.26 and 0.40, respectively, and with those from creatinine-adjusted urinary excretion were 0.22 and 0.33, respectively (21). These correlation coefficients are considered acceptable (22). With regard to the reproducibility of estimations between two questionnaires administered 1 year apart, respective correlation coefficients for the energy-adjusted intake of miso soup, soy food, daidzein, and genistein were 0.80, 0.64, 0.75, and 0.75 for cohort I and 0.75, 0.57, 0.53, and 0.51 for cohort II (23-25).

Among the 46,001 men who responded to the questionnaire, 2,492 who reported extreme total energy intake (<800 or >4,000 kcal) were excluded, leaving 43,509 men for analysis.

Follow-up. Subjects were followed from the 5-year follow-up survey until December 31, 2004. Changes in residence status, including survival, were identified annually through the residential registry in each area or, for those who had moved out of the study area, through the municipal office of the area to which they had moved. Generally, mortality data for residents included in the residential registry are forwarded to the Ministry of Health, Labour, and Welfare and coded for inclusion in the national Vital Statistics. Residency and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are believed to be complete. Here, information on the cause of death was based on death certificates from the respective public health center for those who had not moved out of the original area. Among questionnaire respondents to the 5-year follow-up FFQ, 3,855 men (8.4%) died, 1,492 men (3.2%) moved out of the study area, and 66 men (0.1%) were lost to follow-up during the study period.

The occurrence of cancer was identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the cancer registries. Cases were coded using the International Classification of Diseases for Oncology, Third Edition (26). Death certificate information was used as a supplementary information source. The proportion of cases of prostate cancer first notified by death certificate was 0.9%. The ratio of incidence to mortality was 7.7. The registration rate as introduced by Parkin et al. (27) was 94.3%. The proportion of case patients with prostate cancer ascertained by death certificate only was 0.6%. These ratios were considered satisfactory for the present study. A total of 307 newly diagnosed prostate cancer cases were identified by December 31, 2004.

Finally, a population-based cohort of 43,509 men (18,105 in cohort I and 25,404 in cohort II) was established for analysis. During the 325,371 person-years of follow-up (167,611 in cohort I and 157,760 in cohort II), 307 cases of prostate cancer were newly diagnosed (156 in cohort I and 151 in cohort II).

Statistical Analysis. Person-years of follow-up were calculated for each man from the date of completion of the 5-year follow-up FFQ to the date of prostate cancer diagnosis, the date of emigration from the study area, or the date of death, whichever came first; or if none of these occurred, follow-up was through to the end of the study period (December 31, 2004). Men who were lost to follow-up were censored at the last confirmed date of presence in the study area. The crude incidence rate for prostate cancer was calculated by dividing the number of prostate cancer cases by the number of person-years. The relative risks (RR) of prostate cancer were calculated in quartile for the categories of miso soup consumption, soy food consumption, and isoflavone intake, with the lowest consumption category as the reference. RRs and 95% confidence intervals (95% CI) were calculated by the Cox proportional hazards model, adjusting for age at 5-year follow-up survey and study area (10 public health center areas) according to the SAS PHREG procedure (version 9.1; SAS Institute, Inc., Cary, NC). For further adjustment, additional possible confounders were incorporated into the model: smoking status (never, former, and current); alcohol intake (almost never, <3-4 days/wk, and >5 days/wk); marital status (yes/no); body mass index; and consumption of dairy foods, vegetables, fruit, and total fatty acids.

We conducted additional analyses according to the stage of prostate cancer. Advanced cases were defined by a diagnosis of extraprostatic or metastatic cancer involving lymph nodes or other organs. If this information was not available, advanced cases were defined as those with a high Gleason score (8-10) or poor differentiation. These criteria were selected to allow the identification of advanced cases with a high likelihood of poor prognosis. The remaining cases were organ localized. In this study, there were 74 advanced cases, 220 localized cases, and 13 (4% of total) cases of undetermined stage.

The trend was assessed by assigning ordinal values for categorical variables. All Ps were two sided, and statistical significance was determined at the $P < 0.05$ level.

Results

Distribution of subject characteristics at the 5-year follow-up survey according to quartile of energy-adjusted isoflavone consumption is shown in Table 1, in which the results for genistein were used as a surrogate for isoflavones owing to the high correlation among results for genistein, daidzein, miso soup, and soy food. Men with high genistein consumption were slightly older. Body mass index was higher in the highest category than in the other categories. The proportion of current smokers increased as genistein intake increased, whereas alcohol intake decreased. The proportion of men who live with their wife was lower in the lowest category than in the other categories. Screening-detected prostate cancer accounted for >40% of cases in all categories. Dairy food, fruit, vegetable, and total fatty acid consumption were positively correlated with genistein intake. As expected, soy food and miso soup increased as genistein intake increased and were highly correlated with it ($P < 0.0001$).

Table 2 shows age- and area-adjusted and multivariable RRs and 95% CIs for total prostate cancer by each quartile of genistein, daidzein, miso soup, and soy food consumption. Genistein, daidzein, and soy food consumption slightly decreased the risk of total prostate cancer. Multivariable RRs for the highest versus lowest quartile of genistein, daidzein, and soy food consumption were 0.71 (95% CI, 0.48-1.03), 0.77 (95% CI, 0.52-1.13), and 0.82 (95% CI, 0.57-1.19), respectively. Tests for linear trends were not statistically significant. No statistically significant association was seen between miso soup consumption and total prostate cancer risk (highest versus lowest: RR, 1.04; 95% CI, 0.72-1.50).

We next classified the data according to prostate cancer stage (Table 3). Consumption of genistein decreased the risk of localized prostate cancer in a statistically significant manner (RR, 0.59; 95% CI, 0.38-0.93), although P_{trend} was not statistically significant. Men in the highest quartile of daidzein also had a decreased the risk of localized prostate cancer, although with only marginal significance (RR, 0.66; 95% CI, 0.42-1.04). Miso soup and soy foods tended to decrease the risk of localized prostate cancer, with respective multivariable RRs for the highest versus lowest quartile of 0.78 (95% CI, 0.51-1.20) and 0.77 (95% CI, 0.50-1.19). In contrast, genistein and daidzein increased the risk of advanced prostate cancer, with respective RRs for men with the highest versus lowest consumption of genistein and daidzein of 1.26 (95% CI, 0.56-2.83) and 1.43 (95% CI, 0.63-3.28). Miso soup was dose-dependently increased the risk of advanced prostate cancer, with multivariable RR for the highest versus lowest quartile of 2.79 (95% CI, 1.19-6.55; $P_{trend} = 0.02$). Consumption of soy food was not associated with advanced cancer.

Table 4 shows multivariable RRs and 95% CIs for prostate cancer by stage categorized according to age. We found that the negative association with genistein, daidzein, and soy food and localized prostate cancer became clear when we analysis was restricted to men ages >60 years. Genistein, daidzein, and soy food were dose-dependently associated with a decreased risk of localized prostate cancer, with multivariable RR for the highest versus lowest quartile of 0.52 for genistein (95% CI, 0.30-0.90; $P_{trend} = 0.03$), 0.50 for daidzein (95% CI, 0.28-0.88; $P_{trend} = 0.04$), and 0.52 for soy food (95% CI, 0.29-0.90; $P_{trend} = 0.01$). Miso soup also decreased the risk of localized prostate cancer, although without statistical significance. In contrast, RRs of daidzein and miso soup showed an increased risk of advanced prostate cancer in men ages >60 years old. In particular, miso soup was positively associated with advanced cancer, with a multivariable RR for the highest versus lowest quartile of 2.86 (95% CI, 1.01-8.11). Daidzein tended to increase the risk of advanced cancer (highest versus lowest: RR, 1.49; 95% CI, 0.55-4.03). Genistein was not associated with advanced cancer. Soy food tended to be negatively associated with advanced cancer in men ages >60 years. In men ages ≤60 years, genistein, daidzein, and soy food increased the risk of both localized and advanced prostate cancer. Multivariable RR for the highest versus lowest quartile was 1.18 for genistein, 1.38 for daidzein, and 1.38 for soy food in localized prostate cancer and 2.00 for genistein, 2.46 for daidzein, and 1.48 for soy food in advanced prostate cancer. Miso soup was not associated with localized prostate cancer (RR, 0.82) but was associated with an increased risk of advanced prostate cancer (RR, 2.65). However, none of these values was statistically significant.

To weaken the influence of localized prostate cancer detected by prostate-specific antigen screening, we also analyzed the association between prostate cancer and the four items after excluding screening-detected tumors by stage in men ages >60 years, notwithstanding that screening information was available for only 70% of subjects (Table 5). Results in both localized and advanced prostate cancer were similar to those in Table 4 when screening-detected prostate cancer was included, although the statistical significance in these results was lost. Genistein, daidzein, and miso soup tended to decrease the risk of localized prostate cancer (highest versus lowest: RRs of genistein, daidzein, miso soup, and soy food of 0.52, 0.49, 0.73, and 0.51, respectively) but without statistical significance. In advanced prostate cancer, multivariable RR for the highest versus lowest quartile was 0.85 for genistein,

Table 1. Characteristics of study subjects according to genistein consumption

	Genistein consumption				$P_{difference}$*
	Lowest	Second	Third	Highest	
Age ± SD (y)	56.2 ± 8.2	56.5 ± 7.9	56.7 ± 7.7	57.7 ± 7.6	<0.0001
Body mass index ± SD (kg/m^2)	23.6 ± 3.0	23.6 ± 2.9	23.6 ± 2.8	23.7 ± 2.9	0.009
Current smoker (%)	49.8	52.3	54.5	58.8	<0.0001
Alcohol intake, ≧5 d/wk (%)	49.1	49.7	49.5	46.2	<0.0001
Men who live with their wife (%)	81.1	83.9	83.8	83.2	<0.0001
Screening-detected tumors (%)	40.4	43.9	47.4	42.6	0.97
Dairy food (g/d)	161.2 ± 260.1	159.5 ± 215.1	164.7 ± 200.4	170.1 ± 188.5	0.002
Fruits (g/d)	152.3 ± 169.5	175.7 ± 168.2	193.5 ± 177.6	201.4 ± 177.2	<0.0001
Vegetables (g/d)	163.9 ± 141.6	192.0 ± 143.2	209.5 ± 146.1	225.2 ± 159.7	<0.0001
Total fatty acids (g/d)	48.7 ± 26.9	48.9 ± 23.8	51.0 ± 24.2	51.6 ± 23.0	<0.0001
Soy food (g/d)	33.0 ± 18.8	62.4 ± 26.4	92.3 ± 39.6	164.5 ± 144.6	<0.0001
Miso soup (mL/d)	51.3 ± 39.6	167.4 ± 57.3	301.8 ± 74.2	449.6 ± 139.0	<0.0001
Genistein (mg/d)	8.5 ± 4.3	17.0 ± 5.7	26.8 ± 9.1	49.1 ± 30.9	<0.0001
Daidzein (mg/d)	5.4 ± 2.8	10.8 ± 3.5	16.8 ± 5.5	29.9 ± 17.8	<0.0001

NOTE: Results for genistein are reported as isoflavones because the intake estimates for genistein and daidzein were highly correlated ($P < 0.0001$).
*$P_{difference}$ values of characteristics between categories of genistein consumption were calculated by ANOVA and the χ^2 test for homogeneity.

Table 2. RRs and 95% CIs for total prostate cancer according to quartile of energy-adjusted intake of genistein, daidzein, miso soup, and soy food

	Intake by quartile				P_{trend}
	Lowest (<13.2 mg/d)	Second (13.2-21.2 mg/d)	Third (21.3-32.7 mg/d)	Highest (≧ 32.8 mg/d)	
Genistein					
No. cases	75	76	91	65	
Person-years of follow-up	78,439	81,443	83,208	82,282	
Age/area-adjusted RR (95% CI)	1.00	0.92 (0.67-1.27)	1.16 (0.84-1.59)	0.80 (0.56-1.14)	0.48
Multivariate RR (95% CI)	1.00	0.81 (0.62-1.23)	1.13 (0.81-1.57)	0.71 (0.48-1.03)	0.22
	Intake by quartile				P_{trend}
	Lowest (<8.5 mg/d)	Second (8.5-13.4 mg/d)	Third (13.5-20.3 mg/d)	Highest (≧ 20.4 mg/d)	
Daidzein					
No. cases	70	79	93	65	
Person-years of follow-up	78,260	81,548	83,193	82,370	
Age/area-adjusted RR (95% CI)	1.00	1.01 (0.73-1.40)	1.25 (0.91-1.72)	0.87 (0.61-1.25)	0.77
Multivariate RR (95% CI)	1.00	0.95 (0.67-1.33)	1.21 (0.87-1.70)	0.77 (0.52-1.13)	0.43
	Intake by quartile				P_{trend}
	Lowest (<110.0 mL/d)	Second (110.0-225.9 mL/d)	Third (226.0-355.9 mL/d)	Highest (≧ 356.0 mL/d)	
Miso soup					
No. cases	58	79	85	85	
Person-years of follow-up	75,651	79,621	84,403	85,696	
Age/area-adjusted RR (95% CI)	1.00	1.11 (0.79-1.57)	1.10 (0.78-1.56)	1.06 (0.75-1.51)	0.82
Multivariate RR (95% CI)	1.00	1.10 (0.77-1.58)	1.08 (0.75-1.55)	1.04 (0.72-1.50)	0.94
	Intake by quartile				P_{trend}
	Lowest (<46.6g/d)	Second (46.6-71.8 g/d)	Third (71.9-107.3 g/d)	Highest (≧ 107.4 g/d)	
Soy food					
No. cases	66	88	79	74	
Person-years of follow-up	77,756	81,557	83,116	82,941	
Age/area-adjusted RR (95% CI)	1.00	1.18 (0.85-1.63)	1.05 (0.75-1.47)	0.91 (0.65-1.29)	0.43
Multivariate RR (95% CI)	1.00	1.10 (0.78-1.54)	0.95 (0.67-1.36)	0.82 (0.57-1.19)	0.18

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, body mass index, and intake of total fatty acids, dairy, vegetables, and fruits.

1.10 for daidzein, 1.97 for miso soup, and 0.73 for soy food; however, these results were not statistically significant. Results in subjects ages ≤60 years were similar to those which included screening-detected cancers. Multivariable RR for the highest versus lowest quartile was 1.28 (95% CI, 0.33-4.97) for genistein, 1.55 (95% CI, 0.42-5.78) for daidzein, and 1.94 (95% CI, 0.58-6.52) for soy food in localized prostate cancer and 2.22 (95% CI, 0.50-9.91) for genistein, 2.93 (95% CI, 0.53-16.29) for daidzein, and 1.82 (95% CI, 0.33-9.91) for soy food in advanced prostate cancer. However, these values were not statistically significant (data not shown).

Discussion

In the present study, we observed a dose-dependent decrease in the risk of localized prostate cancer with isoflavone consumption. Men with the highest intake of isoflavones (as genistein, ≧32.8 mg/d) had a decreased risk of prostate cancer compared with those with the lowest intake of isoflavones (as genistein, <13.2 mg/d). To our knowledge, this is the first prospective study to report an inverse association between isoflavone and localized prostate cancer in Japanese, whose intake of soy food is high.

Our results support previous studies, which reported that soy food is protective for prostate cancer. Among case-control studies, Sonoda et al. (17) reported that *natto* (fermented soy) consumption showed a significantly decreasing linear trend for risk of prostate cancer in Japanese; Lee et al. (13) found that the highest intake of tofu and genistein had a statistically significant association with a decreased risk of prostate cancer in Chinese compared with the lowest intake; and Strom et al. (11) reported an inverse association between daidzein intake and prostate cancer risk in American men. Soy foods were also inversely related to prostate cancer in a large multicenter case-control study (12). In prospective studies, Jacobson et al. (10) reported that frequent consumption of soy milk was associated with a decreased risk of prostate cancer in Californian Adventist men. However, no association was seen between tofu consumption and a decreased risk of prostate cancer in Japanese men living in Hawaii (16), nor was tofu or miso soup significantly associated with prostate cancer risk in native Japanese (14). The reason these studies did not show a protective effect of soy foods on prostate cancer may have been due to their evaluation of a single soy food only or their failure to assess specific nutrients such as genistein or daidzein.

Studies *in vivo* and *in vitro* experiments have also shown a protective effect of isoflavones against prostate cancer development. Isoflavones possess weak estrogen activity, inhibit tyrosine protein kinases and angiogenesis, and reduce serum testosterone levels (6, 28, 29). Isoflavones also inhibit 5α-reductase, an enzyme that metabolizes testosterone to dihydrotestosterone (30). Any or all of these mechanisms may explain the inverse association between isoflavones and localized prostate cancer seen here. Moreover, our results are plausible because the incidence of prostate cancer in Japanese is much lower than in Western men (1).

However, when the data were analyzed by stage, we found that the results differed between advanced and localized

cancer. These results suggest that the effects of isoflavone may differ according to stage. One mechanism by which isoflavones reduce the risk of prostate cancer seems to involve estrogen receptor β in prostate tissue (31), but cancer with higher metastatic potential is associated with the complete or partial loss of estrogen receptor β expression (32-34). Moreover, animal studies in rats showed that the beneficial effects of a soy diet play a role in the early stages of tumor development but have no effect in invasive prostate cancer (35, 36). On this basis, isoflavones may prevent the early stages of prostate cancer development only. Clinically significant localized prostate cancer likely arises from latent cancer and then develop to advanced cancer with high mortality (4, 37). Given that the incidence of latent prostate cancer in Japanese men is the same as in Western men despite a lower incidence of prostate cancer (1, 4, 5), isoflavone may delay the progression of latent prostate cancer.

When we limited analysis to men ages >60 years, the association between isoflavone and localized prostate cancer was strengthened. Hoffman et al. (38) reported that men with cancers detected by prostate-specific antigen screening were more often younger than those men in whom cancer was clinically diagnosed. Our study also showed that the proportion of screening-detected cancers was higher (54.6%) in those men ages ≤60 years than in those ages >60 years (28.1%), although prostate-specific antigen screening information was available for only 70% of subjects. However, although we analyzed the association between localized prostate cancer and isoflavones after excluding screening-detected tumors, results did not change. Isoflavone may be protective for localized prostate cancer only in men ages >60 years and may not have a protective effect in the early stage of prostate cancer in younger men.

Our study has several methodologic strengths. First, it was a prospective design, which diminishes the probability of recall bias that is inherent to case-control studies. Second, we evaluated isoflavone intake using a validated questionnaire, and participants had a large variation in isoflavone consumption. One reason for the inconsistent findings for the association between soy food and prostate cancer in previous studies may be errors in exposure measurements and the small exposure variation in Western subjects. Third, we adjusted possible confounding factors to remove associations with other substances. It is also possible that a lifestyle associated with a high intake of soy food may have contributed to the risk of prostate cancer. In this study, the associations between isoflavones and prostate cancer were strengthened after

Table 3. RRs and 95% CIs for prostate cancer according to quartile of energy-adjusted intake of genistein, daidzein, miso soup, and soy food by stage

	Intake by quartile				P_{trend}
	Lowest	Second	Third	Highest	
Localized prostate cancer					
Genistein					
No. cases	54	51	73	42	
Person-years of follow-up	78,329	81,340	83,086	82,219	
Age/area–adjusted RR (95% CI)	1.00	0.81 (0.55-1.20)	1.21 (0.84-1.74)	0.67 (0.44-1.03)	0.31
Multivariate RR (95% CI)	1.00	0.77 (0.52-1.15)	1.16 (0.79-1.69)	0.59 (0.38-0.93)	0.15
Daidzein					
No. cases	51	54	71	44	
Person-years of follow-up	78,150	81,441	83,686	82,296	
Age/area–adjusted RR (95% CI)	1.00	0.90 (0.61-1.32)	1.22 (0.84-1.77)	0.75 (0.49-1.15)	0.50
Multivariate RR (95% CI)	1.00	0.86 (0.57-1.28)	1.20 (0.82-1.76)	0.66 (0.42-1.04)	0.27
Miso soup					
No. cases	44	55	65	56	
Person-years of follow-up	75,540	79,526	84,287	85,620	
Age/area–adjusted RR (95% CI)	1.00	0.96 (0.65-1.43)	0.99 (0.67-1.46)	0.83 (0.55-1.25)	0.40
Multivariate RR (95% CI)	1.00	0.95 (0.63-1.43)	0.98 (0.65-1.46)	0.78 (0.51-1.20)	0.29
Soy food					
No. cases	46	64	58	52	
Person-years of follow-up	77,644	81,454	83,017	82,857	
Age/area–adjusted RR (95% CI)	1.00	1.17 (0.80-1.72)	1.05 (0.70-1.55)	0.88 (0.58-1.32)	0.38
Multivariate RR (95% CI)	1.00	1.06 (0.71-1.57)	0.93 (0.61-1.40)	0.77 (0.50-1.19)	0.17
Advanced prostate cancer					
Genistein					
No. cases	16	23	15	20	
Person-years of follow-up	78,416	81,440	83,193	82,264	
Age/area–adjusted RR (95% CI)	1.00	1.46 (0.77-2.78)	1.02 (0.49-2.11)	1.32 (0.65-2.67)	0.69
Multivariate RR (95% CI)	1.00	1.60 (0.78-3.30)	1.11 (0.50-2.48)	1.26 (0.56-2.83)	0.88
Daidzein					
No. cases	14	23	18	19	
Person-years of follow-up	78,243	81,537	83,185	82,347	
Age/area–adjusted RR (95% CI)	1.00	1.66 (0.85-3.25)	1.40 (0.68-2.88)	1.47 (0.70-3.09)	0.45
Multivariate RR (95% CI)	1.00	1.67 (0.79-3.52)	1.39 (0.63-3.10)	1.43 (0.63-3.28)	0.58
Miso soup					
No. cases	11	18	18	27	
Person-years of follow-up	75,639	79,620	84,382	85,671	
Age/area–adjusted RR (95% CI)	1.00	1.51 (0.70-3.23)	1.58 (0.72-3.44)	2.21 (1.05-4.66)	0.04
Multivariate RR (95% CI)	1.00	1.73 (0.73-4.12)	1.65 (0.67-4.04)	2.79 (1.19-6.55)	0.02
Soy food					
No. cases	16	20	19	19	
Person-years of follow-up	77,736	81,557	83,096	82,923	
Age/area–adjusted RR (95% CI)	1.00	1.21 (0.62-2.35)	1.15 (0.58-2.27)	1.07 (0.53-2.15)	0.92
Multivariate RR (95% CI)	1.00	1.36 (0.65-2.85)	1.19 (0.55-2.56)	1.05 (0.47-2.34)	0.92

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, body mass index, and intake of total fatty acids, dairy, vegetables, and fruits.

Table 4. RRs and 95% CIs for prostate cancer according to quartile of energy-adjusted intake of genistein, daidzein, miso soup, and soy food by stage categorized according to age

	Intake in quartile				P_{trend}
	Lowest	Second	Third	Highest	
>60 y					
Localized prostate cancer					
Genistein					
No. cases	42	37	38	27	
Person-years of follow-up	24,531	25,312	25,859	25,538	
Multivariate RR (95% CI)	1.00	0.81 (0.51-1.29)	0.79 (0.49-1.28)	0.52 (0.30-0.90)	0.03
Daidzein					
No. cases	41	36	42	25	
Person-years of follow-up	24,475	25,398	25,790	25,576	
Multivariate RR (95% CI)	1.00	0.80 (0.50-1.28)	0.90 (0.56-1.45)	0.50 (0.28-0.88)	0.04
Miso soup					
No. cases	34	32	44	34	
Person-years of follow-up	23,842	24,855	26,264	26,279	
Multivariate RR (95% CI)	1.00	0.72 (0.43-1.18)	0.87 (0.54-1.41)	0.65 (0.39-1.11)	0.22
Soy food					
No. cases	35	47	37	25	
Person-years of follow-up	24,270	25,488	25,866	25,616	
Multivariate RR (95% CI)	1.00	1.12 (0.71-1.79)	0.76 (0.46-1.27)	0.52 (0.29-0.90)	0.01
Advanced prostate cancer					
Genistein					
No. cases	15	15	11	12	
Person-years of follow-up	24,585	25,381	25,924	25,572	
Multivariate RR (95% CI)	1.00	1.16 (0.51-2.64)	0.82 (0.33-2.07)	1.03 (0.41-2.59)	0.87
Daidzein					
No. cases	11	19	11	12	
Person-years of follow-up	24,536	25,465	25,852	25,611	
Multivariate RR (95% CI)	1.00	1.97 (0.83-4.66)	1.16 (0.43-3.12)	1.49 (0.55-4.03)	0.75
Miso soup					
No. cases	9	15	13	16	
Person-years of follow-up	23,915	24,904	26,314	26,329	
Multivariate RR (95% CI)	1.00	2.01 (0.74-5.45)	1.79 (0.63-5.13)	2.86 (1.01-8.11)	0.07
Soy food					
No. cases	14	18	11	10	
Person-years of follow-up	24,332	25,539	25,933	25,659	
Multivariate RR (95% CI)	1.00	1.49 (0.66-3.34)	1.06 (0.43-2.54)	0.70 (0.25-1.95)	0.38
≦60 y					
Localized prostate cancer					
Genistein					
No. cases	14	19	24	19	
Person-years of follow-up	53,645	55,739	57,270	57,080	
Multivariate RR (95% CI)	1.00	1.10 (0.55-2.22)	1.51 (0.76-2.98)	1.18 (0.57-2.45)	0.49
Daidzein					
No. cases	14	21	20	21	
Person-years of follow-up	53,555	55,728	57,310	57,310	
Multivariate RR (95% CI)	1.00	1.17 (0.58-2.39)	1.44 (0.71-2.93)	1.38 (0.67-2.83)	0.32
Miso soup					
No. cases	14	20	24	18	
Person-years of follow-up	51,470	54,766	57,743	59,756	
Multivariate RR (95% CI)	1.00	0.96 (0.48-1.92)	1.02 (0.52-2.00)	0.82 (0.40-1.68)	0.62
Soy food					
No. cases	13	20	22	21	
Person-years of follow-up	52,997	55,845	57,205	57,687	
Multivariate RR (95% CI)	1.00	1.17 (0.58-2.39)	1.44 (0.71-2.93)	1.38 (0.67-2.83)	0.32
Advanced prostate cancer					
Genistein					
No. cases	4	5	3	9	
Person-years of follow-up	53,692	55,772	57,296	57,102	
Multivariate RR (95% CI)	1.00	1.05 (0.28-4.00)	0.44 (0.08-2.53)	2.00 (0.53-7.51)	0.32
Daidzein					
No. cases	3	5	5	8	
Person-years of follow-up	53,598	55,772	57,339	57,153	
Multivariate RR (95% CI)	1.00	1.42 (0.33-6.05)	1.21 (0.26-5.75)	2.46 (0.57-10.60)	0.23
Miso soup					
No. cases	2	4	6	9	
Person-years of follow-up	51,506	54,800	57,775	59,781	
Multivariate RR (95% CI)	1.00	1.66 (0.30-9.14)	1.78 (0.34-9.43)	2.65 (0.54-12.89)	0.20
Soy food					
No. cases	3	5	6	7	
Person-years of follow-up	53,044	55,871	57,239	57,708	
Multivariate RR (95% CI)	1.00	1.27 (0.30-5.42)	1.15 (0.26-5.05)	1.48 (0.35-6.20)	0.63

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, body mass index, and intake of total fatty acids, dairy, vegetables, and fruits.

Table 5. RRs of prostate cancer according to quartile of energy-adjusted intake of genistein, daidzein, miso soup, and soy food after excluding screening-detected tumors by stage in men aged more than 60 years old

	Intake in quartile				P_{trend}
	Lowest	Second	Third	Highest	
>60 y					
Localized prostate cancer					
Genistein					
No. cases	32	42	23	17	
Person-years of follow-up	24,457	25,225	25,766	25,455	
Multivariate RR (95% CI)	1.00	0.73 (0.41-1.29)	0.71 (0.39-1.29)	0.52 (0.27-1.03)	0.07
Daidzein					
No. cases	31	22	28	15	
Person-years of follow-up	24,401	25,313	25,699	25,490	
Multivariate RR (95% CI)	1.00	0.69 (0.39-1.24)	0.92 (0.52-1.61)	0.49 (0.24-1.00)	0.13
Miso soup					
No. cases	25	17	30	24	
Person-years of follow-up	23,766	24,780	26,160	26,196	
Multivariate RR (95% CI)	1.00	0.58 (0.30-1.10)	0.95 (0.54-1.70)	0.73 (0.39-1.38)	0.67
Soy food					
No. cases	25	29	26	16	
Person-years of follow-up	24,199	25,383	25,782	25,538	
Multivariate RR (95% CI)	1.00	1.02 (0.58-1.81)	0.79 (0.43-1.46)	0.51 (0.26-1.01)	0.04
Advanced prostate cancer					
Genistein					
No. cases	18	14	10	11	
Person-years of follow-up	24,501	25,276	25,826	25,474	
Multivariate RR (95% CI)	1.00	0.97 (0.46-2.03)	0.62 (0.25-1.50)	0.85 (0.35-2.05)	0.50
Daidzein					
No. cases	13	19	11	10	
Person-years of follow-up	24,449	25,360	25,759	25,509	
Multivariate RR (95% CI)	1.00	1.69 (0.79-3.63)	0.96 (0.39-2.39)	1.10 (0.43-2.87)	0.85
Miso soup					
No. cases	12	14	10	17	
Person-years of follow-up	23,817	24,819	26,218	26,224	
Multivariate RR (95% CI)	1.00	1.32 (0.55-3.16)	1.10 (0.43-2.84)	1.97 (0.80-4.86)	0.18
Soy food					
No. cases	14	20	9	10	
Person-years of follow-up	24,246	25,427	25,835	25,569	
Multivariate RR (95% CI)	1.00	1.82 (0.86-3.86)	0.92 (0.37-2.30)	0.73 (0.27-2.00)	0.31

NOTE: Multivariate RRs were adjusted for age, area, smoking status, drinking frequency, marital status, body mass index, and intake of total fatty acids, dairy, vegetables, and fruits.

adjustment for several confounding factors. Fourth, response rate was high (~80%), and the proportion of subjects lost to follow-up was relatively low (0.1%).

On the other hand, the present study had several limitations. One was our inability to distinguish screening-detected cancer from total prostate cancer. It is possible that men who have health check-ups are more health conscious and may consume more soy food. However, such misclassification, if present, would lead to increase the risk of localized prostate cancer. Therefore, this inability to distinguish would not account for the decreased risk of localized prostate cancer. Another limitation was that the number of advanced prostate cancer cases was small. A larger sample size may have detected the positive effects of isoflavones on advanced prostate cancer with greater precision. Moreover, misclassification of exposure due to changes in isoflavone consumption during the study period might have occurred because we used information on consumption obtained at one point only. If present, however, such misclassification would underestimate the true relative risk.

In summary, we found that isoflavone intake was associated with a decreased risk of localized prostate cancer but tended to be associated with an increased risk of advanced prostate cancer. Recent interest has focused on whether isoflavones have chemopreventive effects. Given that Japanese consume isoflavones regularly throughout life, we do not yet know the period during which the effects of isoflavones on prostate cancer are preventive. Further research is required, including well-designed clinical trials in humans.

Appendix A

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References

1. Parkin DM, Whelan SL, Ferlay J, Thomas DB. Cancer incidence in five continents. Vol. VIII. Lyon: IARC; 2002.
2. Tsugane S, de Souza JM, Costa ML, Jr., et al. Cancer incidence rates among Japanese immigrants in the city of Sao Paulo, Brazil, 1969-78. Cancer Causes Control 1990;1:189–93.
3. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 1991;63:963–6.
4. Yatani R, Kusano I, Shiraishi T, Hayashi T, Stemmermann GN. Latent prostatic carcinoma: pathological and epidemiological aspects. Jpn J Clin Oncol 1989;19:319–26.
5. Muir CS, Nectoux J, Staszewski J. The epidemiology of prostatic cancer. Geographical distribution and time-trends. Acta Oncol 1991;30:133–40.
6. Magee PJ, Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr 2004;91:513–31.
7. Morton MS, Arisaka O, Miyake N, Morgan LD, Evans BA. Phytoestrogen concentrations in serum from Japanese men and women over forty years of age. J Nutr 2002;132:3168–71.
8. Adlercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 1993;342:1209–10.
9. Morton MS, Chan PS, Cheng C, et al. Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. Prostate 1997;32:122–8.
10. Jacobsen BK, Knutsen SF, Fraser GE. Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). Cancer Causes Control 1998;9:553–7.
11. Strom SS, Yamamura Y, Duphorne CM, et al. Phytoestrogen intake and prostate cancer: a case-control study using a new database. Nutr Cancer 1999;33:20–5.
12. Kolonel LN, Hankin JH, Whittemore AS, et al. Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. Cancer Epidemiol Biomarkers Prev 2000;9:795–804.
13. Lee MM, Gomez SL, Chang JS, Wey M, Wang RT, Hsing AW. Soy and isoflavone consumption in relation to prostate cancer risk in China. Cancer Epidemiol Biomarkers Prev 2003;12:665–8.
14. Allen NE, Sauvaget C, Roddam AW, et al. A prospective study of diet and prostate cancer in Japanese men. Cancer Causes Control 2004;15:911–20.
15. Jian L, Zhang DH, Lee AH, Binns CW. Do preserved foods increase prostate cancer risk? Br J Cancer 2004;90:1792–5.
16. Nomura AM, Hankin JH, Lee J, Stemmermann GN. Cohort study of tofu intake and prostate cancer: no apparent association. Cancer Epidemiol Biomarkers Prev 2004;13:2277–9.
17. Sonoda T, Nagata Y, Mori M, et al. A case-control study of diet and prostate cancer in Japan: possible protective effect of traditional Japanese diet. Cancer Sci 2004;95:238–42.
18. Otani T, Iwasaki M, Inoue M, Tsugane S. Body mass index, body height, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan public health center-based prospective study. Cancer Causes Control 2005;16:839–50.
19. Kimira M, Arai Y, Shimoi K, Watanabe S. Japanese intake of flavonoids and isoflavonoids from foods. J Epidemiol 1998;8:168–75.
20. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr 2000;130:2243–50.
21. Yamamoto S, Sobue T, Sasaki S, et al. Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a Japanese population in comparison with dietary records and blood and urine isoflavones. J Nutr 2001;131:2741–7.
22. Willett W. Nutritional epidemiology. 2nd ed. New York (NY): Oxford University Press; 1998.
23. Ishihara J, Sobue T, Yamamoto S, et al. Validity and reproducibility of a self-administered food frequency questionnaire in the JPHC Study Cohort II: study design, participant profile and results in comparison with Cohort I. J Epidemiol 2003;13:S134–47.
24. Sasaki S, Ishihara J, Tsugane S. Reproducibility of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess food and nutrient intake. J Epidemiol 2003;13:S115–24.
25. Tsugane S, Kobayashi M, Sasaki S. Validity of the self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I: comparison with dietary records for main nutrients. J Epidemiol 2003;13:S51–6.
26. WHO. International classification of diseases for oncology. 3rd ed. Geneva (Switzerland): WHO; 2000.
27. Parkin DM, Chen VW, Ferlay J, Galceran J, Storm HH, Whelan SL. Comparability and quality control in cancer registration. IARC Technical Report No. 19. Lyon: IARC; 1994.
28. Nagata C, Inaba S, Kawakami N, Kakizoe T, Shimizu H. Inverse association of soy product intake with serum androgen and estrogen concentrations in Japanese men. Nutr Cancer 2000;36:14–8.
29. Holzbeierlein JM, McIntosh J, Thrasher JB. The role of soy phytoestrogens in prostate cancer. Curr Opin Urol 2005;15:17–22.
30. Evans BA, Griffiths K, Morton MS. Inhibition of 5 α-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. J Endocrinol 1995;147:295–302.
31. Ganry O. Phytoestrogens and prostate cancer risk. Prev Med 2005;41:1–6.
32. Horvath LG, Henshall SM, Lee CS, et al. Frequent loss of estrogen receptor-β expression in prostate cancer. Cancer Res 2001;61:5331–5.
33. Leav I, Lau KM, Adams JY, et al. Comparative studies of the estrogen receptors β and α and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. Am J Pathol 2001;159:79–92.
34. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor β (ERβ) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate 2003; 54:79–87.
35. Landstrom M, Zhang JX, Hallmans G, et al. Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. Prostate 1998;36:151–61.
36. Bylund A, Zhang JX, Bergh A, et al. Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. Prostate 2000;42:304–14.
37. Vij U, Kumar A. Phyto-oestrogens and prostatic growth. Natl Med J India 2004;17:22–6.
38. Hoffman RM, Stone SN, Espey D, Potosky AL. Differences between men with screening-detected versus clinically diagnosed prostate cancers in the USA. BMC Cancer 2005;5:27.

ORIGINAL ARTICLE

Effect of Soy Isoflavones on Endometriosis

Interaction With Estrogen Receptor 2 Gene Polymorphism

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Background: Progression of endometriosis is considered estrogen-dependent. Dietary soy isoflavones may affect the risk of endometriosis, and polymorphisms in estrogen receptor genes may modify this association. We examined associations among soy isoflavone intake, estrogen receptor 2 (*ESR2*) gene polymorphisms and risk of endometriosis.

Methods: We recruited women age 20–45 years old who had consulted a university hospital for infertility in Tokyo, Japan in 1999 or 2000. A total of 138 eligible women were diagnosed laparoscopically and classified into 3 subgroups: control (no endometriosis), early endometriosis (stage I–II) and advanced endometriosis (stage III–IV). We measured urinary levels of genistein and daidzein as markers for dietary intake of soy isoflavones, and genotyped *ESR2* gene *Rsa*I polymorphisms.

Results: Higher levels of urinary genistein and daidzein were associated with decreased risk of advanced endometriosis (P for trend = 0.01 and 0.06, respectively) but not early endometriosis. For advanced endometriosis, the adjusted odds ratio for the highest quartile group was 0.21 (95% confidence interval = 0.06–0.76) for genistein and 0.29 (0.08–1.03) for daidzein, when compared with the lowest group. Inverse associations were also noted between urinary isoflavones and the severity of endometriosis (P for trend = 0.01 for genistein and 0.07 for daidzein). For advanced endometriosis, *ESR2* gene *Rsa*I polymorphism appeared to modify the effects of genistein (P for interaction = 0.03).

Conclusions: Dietary isoflavones may reduce the risk of endometriosis among Japanese women.

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Soy isoflavones are phytoestrogens found in soybeans. Phytoestrogens are plant-derived nonsteroidal compounds that possess estrogen-like biologic activities. These compounds reportedly display weak estrogenic and antiestrogenic properties.[1–3] The 2 primary isoflavones found in soy are genistein and daidzein. Structural similarities allow isoflavones to bind to estrogen receptors.[4]

It has been hypothesized that soy isoflavones may play a role in the etiology of estrogen-related diseases and several epidemiologic studies have been conducted; however, findings have been complicated and inconsistent.[5–7] A prospective study in Japan, where isoflavone intake is known to be relatively high, showed a protective effect on postmenopausal breast cancer.[5] On the other hand, a nested case–control study in the United Kingdom, where intake is relatively low, showed that serum and urinary isoflavone levels were associated with increased breast cancer risk.[6] A recent meta-analysis found a small reduction in breast cancer risk associated with soy intake.[7] However, the authors suggested that the results should be interpreted cautiously due to potential exposure misclassification, confounding, lack of a dose-response pattern and the possibility of adverse effects of soy constituents.

Endometriosis is a benign, proliferative disease in which tissue similar to endometrial tissue is found outside the uterus—usually in the pelvic cavity, but sometimes in distant organs. Endometriosis is commonly accompanied by pelvic pain and infertility. Both genetic and environmental factors may contribute.[8] The reported prevalence of largely asymptomatic endometriosis found in women undergoing tubal ligation is about 4%, ranging from 1% to 7%.[9] Progression of endometriosis is considered estrogen-dependent.[10] Soy isoflavones might thus be expected to affect the risk and severity of endometriosis. However, few studies have investigated the effects of soy isoflavones on endometriosis.

Several studies have recently described associations between estrogen receptor (ESR) gene polymorphisms and endometriosis.[11–13] Genistein and daidzein reportedly display much greater affinity for ESR2 than for ESR1,[14] suggesting

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that the estrogenic or antiestrogenic properties of soy isoflavones may occur preferentially through ESR2. Although functional variability of *ESR2* gene polymorphisms could feasibly be associated with response to soy isoflavones, whether *ESR2* gene polymorphisms exert altered phenotypic effects on endometriosis through interactions with soy isoflavones is not known.

The present study investigated whether urinary genistein and daidzein are associated with risk and severity of endometriosis, and whether polymorphisms in the *ESR2* gene are associated with response to soy isoflavones.

METHODS

Study Protocol and Ethics

This study was part of a case–control study conducted on a Japanese population to investigate associations between genetic and environmental factors in endometriosis.[15] We recruited consecutive female patients age 20 to 45-year-old who attended the Department of Obstetrics and Gynecology at Jikei University School of Medicine Hospital for infertility in 1999 or 2000. Since pregnancy commonly results in complete resolution of minimal or mild endometriosis, women who had given birth or lactated were ineligible, leaving a total of 159 women who met the criteria. After excluding 15 women who did not give consent, 5 who did not undergo blood screening or laparoscopic examination, and 1 whose DNA sample was not available, a total of 138 women were available for the study (participation rate = 87%). No participants had undergone therapy before laparoscopic examination.

All study protocols were approved by the Institutional Review Boards of Jikei University, National Cancer Center and National Institute for Environmental Studies. All participants provided written informed consent before laparoscopic examination.

Before the laparoscopic examination, participants were interviewed by a single trained interviewer using a structured questionnaire to collect information on demographic factors, age, height, weight, medical history for themselves and their families, reproductive and menstrual history, oral contraceptive use, food- and alcohol-consumption frequency, and smoking history.

Participants collected first morning urine sample using a paper cup and plastic tube, and gave a fasting blood sample before the laparoscopic examination. Blood samples were divided into plasma and buffy layers. All biologic samples were stored at 80°C until analysis.

Diagnosis of Endometriosis

Laparoscopy is necessary for definitive diagnosis of endometriosis. In the present study, all participants underwent diagnostic laparoscopy, and stage of endometriosis was determined by trained gynecologists in accordance with the revised classifications of the American Fertility Society.[16] Endometriosis was absent in 59 women (43%), Stage I in 21 women (15%), Stage II in 10 women (7%), Stage III in 23 women (17%) and Stage IV in 25 women (18%). Current theories of endometriosis suggest that what is defined as minimal/mild endometriosis may actually represent a normal physiologic process. Furthermore, a lack of consistency between laparoscopic and histologic diagnosis has been reported, particularly for minimal/mild endometriosis.[17] Considering the more severe stages as a separate category thus appears logical.[18] Based on surgically or pathologically confirmed disease status, we classified cases into 2 subgroups: early (Stage I–II) or advanced endometriosis (stage III–IV). Women without endometriosis were defined as controls.

Determination of Soy Isoflavone Levels

Urinary levels of soy isoflavones offer a useful biomarker for dietary intake and plasma concentration of isoflavones.[19–21] The present study measured urinary levels of genistein and daidzein as markers for dietary intake of soy isoflavones. A total of 30 mL of first-morning urine was collected before laparoscopic examination. Genistein and daidzein levels were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the modified methods of Gamache and Acworth.[22]

Concentrations of genistein and daidzein were determined by linear regression of peak height for each standard, and were adjusted according to recovery rate of the internal standard. The regression coefficient of peak height and concentration calculated for soy isoflavones revealed a linearity range of 0–8.0 μg/mL, with correlation coefficient values >0.995. Voltametric response for the standard solution displayed coefficients of variation of 2.7%–8.4% for intraday variation and 11.1%–12.2% for interday variation. Recovery rates of soy isoflavones in urine samples ranged between approximately 85% and 100%. Detection limits were 3.22 ng/mL for genistein and 4.14 ng/mL for daidzein.

Concentrations of urinary genistein and daidzein were adjusted by urinary creatinine concentration to correct for variability in urine dilution (μmol/g Cre). All measurements were performed by investigators blinded to case–control status.

Genotyping of *ESR2* Gene Polymorphism

The *ESR2 Rsa*I polymorphism, comprising a G-to-A change at nucleotide 1082 in exon 5, was genotyped using polymerase chain reaction (PCR) restriction fragment length polymorphism methods.[23] Blood samples were obtained before laparoscopic examination. Genomic DNA samples were extracted from peripheral white blood cells using a standard protease K method. PCR products were digested using 5 U of *Rsa*I restriction enzyme at 37°C for 8 hours, then electrophoresed on a 3% agarose gel containing ethidium bromide.

In this study, *ESR2 Rsa*I polymorphism is represented by the *r* and *R* alleles, with *R* indicating the presence of corresponding restriction sites, and *r* indicating the absence of restriction sites. For quality control, blinded control samples were inserted to validate genotyping identification procedures. Concordance for blinded samples was 100%. Genotyping was conducted by investigators blinded to case–control status.

Statistical Analysis

To assess differences between cases and controls, basic characteristics and possible risk factors for endometriosis were compared using Student *t* test and the χ^2 test. Spearman correlation coefficients between urinary level of genistein and daidzein were calculated. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for urinary levels by an unconditional logistic regression model following classification into medians or quartiles based on control distribution. Linear trends for ORs were tested in the unconditional logistic regression model by treating the categories as ordinal variables. We evaluated trends for median values according to disease stage to assess associations between urinary levels of genistein and daidzein and disease stage.

To compare observed and expected genotype frequencies, we tested for Hardy-Weinberg equilibrium by using an exact test. *ESR2 Rsa*I polymorphism was classified into 2 subgroups according to the presence of corresponding restriction sites: *r/r* genotype; and *R/r* + *R/R* genotype. ORs and 95% CIs were calculated for associations between *ESR2 Rsa*I polymorphism and endometriosis using the unconditional logistic regression model.

To investigate whether the *ESR2 Rsa*I genotype modified the effect of urinary levels of genistein or daidzein, we calculated ORs and 95% CIs of endometriosis according to a combination of subgroups for the *ESR2 Rsa*I genotype and urinary isoflavones, using the unconditional logistic regression model. A low level of urinary isoflavones in combination with *R/r* + *R/R* genotype was considered as the reference group. Interactions between *ESR2 Rsa*I polymorphism and urinary isoflavones in the risk of endometriosis were tested with the Wald test using product terms between urinary genistein or daidzein and genotypes.

The present study was designed to have 80% power to detect a decrease in risk of two-thirds at the 5% level of significance. All statistical tests were based on 2-tailed probabilities. We adjusted ORs and 95% CIs for possible confounding factors of endometriosis, namely age (continuous), menstrual cycle (continuous), and duration of menstrual bleeding (less than 7 days or 7 days or more).[9,10] We used SPSS for Windows software version 11.0 (SPSS JAPAN, Tokyo, Japan) for statistical analyses.

RESULTS

Baseline Characteristics and Possible Risk Factors for Endometriosis

T1 Table 1 shows baseline characteristics and possible risk factors for endometriosis in controls and cases. No important differences in mean age or body mass index were identified between groups. Distribution of menstrual bleeding, hypermenorrhea, and smoking also did not differ substantially. The advanced endometriosis group had a shorter mean menstrual cycle length than controls (controls, 30.7 ± 6.1 days; advanced endometriosis, 28.3 ± 3.0 days) and was more likely to have menstrual cramps and dyspareunia.

TABLE 1. Baseline Characteristics and Possible Risk Factors for Endometriosis

		Endometriosis	
	Controls (n = 59)	Early (Stage I–II) (n = 31)	Advanced (Stage III–IV) (n = 48)
Baseline Characteristics			
Age (yrs); mean ± SD	33.1 ± 4.1	32.3 ± 3.2	32.6 ± 3.7
Body mass index (kg/m^2); mean ± SD	21.0 ± 3.4	20.6 ± 2.1	20.2 ± 2.1
Menstrual cycle (d); mean ± SD	30.7 ± 6.1	29.6 ± 3.6	28.3 ± 3.0
Menstrual bleeding; no. (%)			
<7 days	42 (71)	21 (68)	35 (73)
≥7 days	15 (25)	6 (19)	10 (21)
Missing	2 (3)	4 (13)	3 (6)
Hypermenorrhea; no. (%)			
No	39 (66)	20 (65)	29 (60)
Yes	18 (31)	7 (23)	15 (31)
Missing	2 (3)	4 (13)	4 (8)
Menstrual cramp; no. (%)			
No	10 (17)	2 (6)	1 (2)
Yes	47 (80)	25 (81)	44 (92)
Missing	2 (3)	4 (13)	3 (6)
Dyspareunia; no. (%)			
No	31 (53)	12 (39)	10 (21)
Yes	25 (42)	14 (45)	34 (71)
Missing	3 (5)	5 (16)	4 (8)
Smoking status; no. (%)			
Never	38 (64)	19 (61)	29 (60)
Current, ever smoker	19 (32)	8 (26)	15 (31)
Missing	2 (3)	4 (13)	4 (8)

Effect of Urinary Isoflavones on Endometriosis

Table 2 shows risk of endometriosis according to median or quartile levels of urinary isoflavones. In controls, median isoflavone level was 3.24 μmol/g Cre for genistein and 4.01 μmol/g Cre for daidzein. The Spearman correlation coefficient between genistein and daidzein was 0.84. Urinary genistein and daidzein levels were inversely associated with advanced endometriosis (*P* for trend = 0.01 and 0.06, respectively) but not with early endometriosis. For advanced endometriosis, the adjusted odds ratio for the highest quartile group was 0.21 (95% CI = 0.06–0.76) for genistein and 0.29 (0.08–1.03) for daidzein when compared with the lowest group. T2

Table 3 shows the trends of median values for urinary isoflavones according to disease stage. An inverse relationship with stage of endometriosis was observed for both genistein levels (*P* for trend = 0.01) daidzein levels (*P* for trend = 0.07). T3, AQ: 1

Associations Between *ESR2 Rsa*I Polymorphism and Endometriosis

Table 4 shows the genotypic distribution of *ESR2 Rsa*I polymorphism and associations with risk of endometriosis. T4

TABLE 2. Association between Urinary Isoflavone Level and Risk of Endometriosis

		Endometriosis			
		Early (Stage I–II)		Advanced (Stage III–IV)	
Urinary Isoflavone	Controls	No.	OR (95% CI)*	No.	ORs (95% CI)*
Genistein (μmol/g creatinine)					
<1.60†	14	7	1.00	22	1.00
1.60–3.23	15	12	1.86 (0.49–7.09)	13	0.65 (0.21–2.01)
3.24–6.49	15	7	0.82 (0.18–3.80)	7	0.40 (0.12–1.34)
≥6.50	15	5	0.63 (0.14–2.89)	6	0.21 (0.06–0.76)
P for trend	0.34	0.01			
Low level (<3.24)†	29	19	1.00	35	1.00
High level (≥3.24)	30	12	0.50 (0.18–1.39)	13	0.35 (0.14–0.87)
Daidzein (μmol/g creatinine)					
<1.94†	14	5	1.00	16	1.00
1.94–4.00	15	7	1.87 (0.41–8.57)	15	0.84 (0.26–2.73)
4.01–7.94	15	12	2.16 (0.49–9.41)	10	0.65 (0.20–2.09)
≥7.95	15	7	1.33 (0.30–5.97)	7	0.29 (0.08–1.03)
P for trend	0.73	0.06			
Low level (<4.01)†	29	12	1.00	31	1.00
High level (≥4.01)	30	19	1.21 (0.45–3.27)	17	0.49 (0.21–1.15)

*Adjusted for age (continuous); menstrual cycle (continuous); and duration of menstrual bleeding (less than 7 days or 7 days or more).
†Reference category.

TABLE 3. Median Values of Urinary Isoflavone Level and Stage of Endometriosis

Urinary Isoflavone	Controls (n = 59)	Early (Stage I–II) (n = 31)	Advanced (Stage III–IV) (n = 48)	*P* for Trend*
Genistein (μmol/g creatinine)†	3.2 (1.6–6.5)	2.6 (1.7–5.2)	1.7 (0.6–4.1)	0.01
Daidzein (μmol/g creatinine)†	4.0 (1.9–8.0)	4.9 (2.6–7.6)	2.6 (1.0–5.0)	0.07

*Jonckheere-Terpstra test.
†Median (25th–75th percentile).

The *ESR2 RsaI r/r* genotype was predominant. Allele frequencies of *ESR2 RsaI* polymorphism were 0.77 for the *r* allele and 0.23 for the *R* allele. In addition, the distribution of *ESR2 RsaI* polymorphism was in Hardy–Weinberg equilibrium ($P = 0.26$). The *ESR2 RsaI r/r* genotype was associated with reduced risk of early endometriosis compared with the *R/r* + *R/R* genotype (OR = 0.30; CI = 0.11–0.85). The association was weaker for advanced endometriosis (0.67; 0.29–1.55).

TABLE 4. Association Between *ESR2 RsaI* Polymorphism and Risk of Endometriosis

		Early (Stage I–II)		Advanced (Stage III–IV)	
Genotype*	Controls	No.	ORs (95% CI)†	No.	ORs (95% CI)†
R/r + *R/R*‡	26	21	1.00	26	1.00
r/r	33	10	0.30 (0.11–0.85)	22	0.67 (0.29–1.55)

*Exact test for Hardy–Weinberg equilibrium: $P = 0.26$.
†Adjusted for age (continuous); menstrual cycle (continuous); and duration of menstrual bleeding (less than 7 days or 7 days or more).
‡Reference category.

Interactions Between *ESR2 RsaI* Polymorphism and Urinary Isoflavones in the Risk of Endometriosis

Table 5 shows ORs and 95% CIs of endometriosis for combinations of *ESR2 RsaI* genotype and urinary isoflavone levels. Compared with subjects with the *ESR2 RsaI R/r* + *R/R* genotype and a low genistein level, ORs of advanced endometriosis were lower among the 3 other groups. The adjusted OR was 0.10 (95% CI = 0.02–0.48) for subjects with *ESR2 RsaI R/r* + *R/R* genotype with high genistein level; 0.32 (0.10–1.04) for subjects with *ESR2 RsaI r/r* genotype with low genistein level; 0.27 (0.08–0.92) for

TABLE 5. Interactions Between ESR2 RsaI Polymorphism and Urinary Isoflavone in the Risk of Endometriosis

Genotype	Urinary Isoflavone (μmol/g creatinine)	Controls	Early (Stage I–II)		Advanced (Stage III–IV)	
			No.	ORs (95%CI)*	No.	ORs (95%CI)*
Genistein						
R/r + *R/R*	Low†	11	11	1.00	22	1.00
R/r + *R/R*	High	15	10	0.88 (0.23–3.38)	4	0.10 (0.02–0.48)
r/r	Low	18	8	0.54 (0.15–1.93)	3	0.32 (0.10–1.04)
r/r	High	15	2	NC	9	0.27 (0.08–0.92)
P for interaction				NC		0.03
Daidzein						
R/r + *R/R*	Low†	15	10	1.00	19	1.00
R/r + *R/R*	High	11	11	1.28 (0.33–4.96)	7	0.35 (0.09–1.34)
r/r	Low	14	2	0.18 (0.03–1.09)	12	0.56 (0.18–1.78)
r/r	High	19	8	0.44 (0.12–1.61)	10	0.39 (0.13–1.20)
P for interaction				0.58		0.45

*Adjusted for age (continuous); menstrual cycle (continuous); and duration of menstrual bleeding (less than 7 days or 7 days or more).
†Reference category.
NC, estimates were not calculated due to missing data.

subjects with *ESR2 Rsa*I *r/r* genotype with high genistein level. A significant interaction was noted between *ESR2 Rsa*I polymorphism and genistein levels in risk of advanced endometriosis (*P* for interaction = 0.03). Interactions between *ESR2 Rsa*I polymorphism and genistein level were not observed in early endometriosis. Although a similar pattern was observed for ORs of both early and advanced endometriosis for the combinations of *ESR2 Rsa*I genotype and urinary daidzein level, these may have been due to chance.

DISCUSSION

The present study showed an inverse association between urinary isoflavones and the risk of advanced endometriosis. This association was stronger for genistein than daidzein. In addition, there was statistical evidence for interaction between urinary genistein and *ESR2* gene polymorphisms.

The reduced risk of endometriosis following ingestion of soy isoflavones may be attributable to antiestrogenic properties of these compounds. A previous study showed that prolonged exposure to genistein results in decreased levels of estrogen receptor mRNA in addition to decreased response to estradiol stimulation.[24] Plasma levels of isoflavones can be 10,000- to 100,000-fold higher than those of estradiol.[25] When the relative binding affinity of 17β-estradiol was set at 100 in solid-phase competition experiments, relative binding affinity for ESR2 was 87 for genistein and 0.5 for daidzein.[14] Although the elimination half-life from blood and urine is reportedly 7–8 hours for both genistein and daidzein,[26] long-term soy diets may modify the physiologic effects of estrogens. Given these facts, a lower prevalence of endometriosis might be expected in Japanese populations compared with Western countries, as with breast cancer. Nevertheless, the prevalence of endometriosis in the Japanese general population remains unclear due to the need for surgical diagnosis.

Our finding showed that the strength of association was stronger for genistein than for daidzein. One possible explanation is the difference in their binding affinities to ESR2. A second possibility is based on the difference in metabolism between genistein and daidzein. Daidzein can be metabolized to equol and *O*-desmethylangolites by intestinal bacteria, and these metabolites are absorbed, enter the circulation, and are excreted in urine. Although equol has been suggested to possess stronger estrogenic properties than genistein, some individuals are capable of equol production whereas others are not, probably because of differences in gut microflora. This difference might play a role in the weaker associations for daidzein than genistein.[27]

ESR2 plays important roles in endometrial function, in addition to the well-known role of ESR1 in endometrial proliferation and differentiation.[28] The *ESR2 Rsa*I polymorphism does not cause amino acid changes, but may well be associated with altered ligand-binding affinity or transcriptional activity. Genes containing single nucleotide polymorphisms (SNPs) can cause different structural folds in mRNA,[29] and these mRNA variants may possess different biologic functions during interactions with other cellular components. Altered estrogen or soy isoflavone signal transduction thanks to *ESR2* gene polymorphisms may be directly responsible for interindividual susceptibility to and severity of endometriosis.

The present study found evidence of an interaction between urinary genistein and *ESR2* gene polymorphisms. Isoflavones may play a more effective role among the *ESR2 Rsa*I *R/r* + *R/R* genotype than the *r/r* genotype, although the latter itself is likely to be protective for endometriosis. This result should be interpreted cautiously, however, because of the relatively small number of subjects—a major limitation of this study. When the number of subjects studied is not large

and the expected difference is small, actual differences are quite likely to pass undetected. Inconsistent results between early and advanced endometriosis might be attributable to the lack of sufficient numbers and possible misclassification in the early endometriosis group. Alternatively, the observed interactions may have occurred merely by chance.

A second issue is our definition of cases and controls. In accordance with the revised classifications of the American Fertility Society, we defined women without endometriosis as controls and women with early (Stage I–II) and advanced endometriosis (Stage III–IV) as cases,[16] although there is no clear criterion for dichotomizing cases. The present study did not show a persuasive inverse association between urinary isoflavones and the risk of early endometriosis, although a strong protective effect was found for advanced endometriosis. Further analysis, however, did show an inverse association between urinary isoflavones and the severity of endometriosis. This finding may be reasonable give that endometriosis occurs in a continuum of severity.

A third issue is measurement of urinary levels of isoflavones. The present study measured urinary excretion of genistein and daidzein as markers of soy isoflavone consumption. Urinary excretion of soy isoflavones is reportedly related to annual dietary intake of soy isoflavones.[19] Since we collected spot urine samples, intraindividual variation in urinary isoflavones cannot be ignored. Such misclassification, however, is probably nondifferential and would lead to a null result.

Participants in the present study were infertile. They might therefore have changed their diet due to their symptoms or in attempt to become pregnant. If a change in diet was more likely among patients with advanced endometriosis than the controls, our findings might have been the result of the change in diet. In addition, given reports that factors associated with endometriosis differ between parous women (who experienced neither primary nor secondary infertility) and nulliparous infertile women,[30,31] the influence of urinary isoflavone levels on endometriosis risk between the 2 groups may have differed. Therefore, our present findings may be limited to infertile women.

In conclusion, in a case–control study in infertile Japanese women, we found that higher urinary level of isoflavones was associated with a reduced risk of advanced endometriosis. Although the interaction between urinary genistein and *ESR2* gene polymorphisms supported the mechanism for a role of isoflavones in the etiology of endometriosis, further studies with a large number of subjects are needed to confirm these findings.

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REFERENCES

1. Tham DM, Gardner CD, Haskell WL. Clinical review 97: potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. *J Clin Endocrinol Metab.* 1998;83:2223–2235.
2. Wagner JD, Anthony MS, Cline JM. Soy phytoestrogens: research on benefits and risks. *Clin Obstet Gynecol.* 2001;44:843–852.
3. Gruber CJ, Tschugguel W, Schneeberger C, et al. Production and actions of estrogens. *N Engl J Med.* 2002;346:340–352.
4. Limer JL, Speirs V. Phyto-oestrogens and breast cancer chemoprevention. *Breast Cancer Res.* 2004;6:119–127.
5. Yamamoto S, Sobue T, Kobayashi M, et al. Soy, isoflavones, and breast cancer risk in Japan. *J Natl Cancer Inst.* 2003;95:906–913.
6. Grace PB, Taylor JI, Low YL, et al. Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. *Cancer Epidemiol Biomarkers Prev.* 2004;13:698–708.
7. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. *J Natl Cancer Inst.* 2006;98:459–471.
8. Kennedy S. The genetics of endometriosis. *J Reprod Med.* 1998;43:263–268.
9. Cramer DW, Missmer SA. The epidemiology of endometriosis. *Ann NY Acad Sci.* 2002;955:11–22.
10. Vigano P, Parazzini F, Somigliana E, et al. Endometriosis: epidemiology and aetiological factors. *Best Pract Res Clin Obstet Gynaecol.* 2004;18:177–200.
11. Georgiou I, Syrrou M, Bouba I, et al. Association of estrogen receptor gene polymorphisms with endometriosis. *Fertil Steril.* 1999;72:164–166.
12. Kitawaki J, Obayashi H, Ishihara H, et al. Oestrogen receptor-alpha gene polymorphism is associated with endometriosis, adenomyosis and leiomyomata. *Hum Reprod.* 2001;16:51–55.
13. Wang Z, Yoshida S, Negoro K, et al. Polymorphisms in the estrogen receptor beta gene but not estrogen receptor alpha gene affect the risk of developing endometriosis in a Japanese population. *Fertil Steril.* 2004;81:1650–1656.
14. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology.* 1998;139:4252–4263.
15. Tsukino H, Hanaoka T, Sasaki H, et al. Associations between serum levels of selected organochlorine compounds and endometriosis in infertile Japanese women. *Environ Res.* 2005;99:118–125.
16. American Fertility Society. Revised American Fertility Society classification of endometriosis. *Fertil Steril.* 1985;43:351–352.
17. Marchino GL, Gennarelli G, Enria R, et al. Diagnosis of pelvic endometriosis with use of macroscopic versus histologic findings. *Fertil Steril.* 2005;84:12–15.
18. Zondervan KT, Cardon LR, Kennedy SH. What makes a good case-control study? Design issues for complex traits such as endometriosis. *Hum Reprod.* 2002;17:1415–1423.
19. Maskarinec G, Singh S, Meng L, et al. Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. *Cancer Epidemiol Biomarkers Prev.* 1998;7:613–619.
20. Arai Y, Uehara M, Sato Y, et al. Comparison of isoflavones among dietary intake, plasma concentration and urinary excretion for accurate estimation of phytoestrogen intake. *J Epidemiol.* 2000;10:127–135.
21. Yamamoto S, Sobue T, Sasaki S, et al. Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a japanese population in comparison with dietary records and blood and urine isoflavones. *J Nutr.* 2001;131:2741–2747.
22. Gamache PH, Acworth IN. Analysis of phytoestrogens and polyphenols in plasma, tissue, and urine using HPLC with coulometric array detection. *Proc Soc Exp Biol Med.* 1998;217:274–280.
23. Sundarrajan C, Liao WX, Roy AC, et al. Association between estrogen receptor-beta gene polymorphisms and ovulatory dysfunctions in patients with menstrual disorders. *J Clin Endocrinol Metab.* 2001;86:135–139.
24. Wang TT, Sathyamoorthy N, Phang JM. Molecular effects of genistein on estrogen receptor mediated pathways. *Carcinogenesis.* 1996;17:271–275.
25. Manach C, Williamson G, Morand C, et al. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr.* 2005;81(1 Suppl):230S–242S.

26. King RA, Bursill DB. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. *Am J Clin Nutr*. 1998;67:867–872.
27. Atkinson C, Frankenfeld CL, Lampe JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Exp Biol Med (Maywood)*. 2005;230:155–170.
28. Lecce G, Meduri G. Ancelin M, et al. Presence of estrogen receptor beta in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells. *J Clin Endocrinol Metab*. 2001;86:1379–1386.
29. Shen LX, Basilion JP, Stanton VP Jr. Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proc Natl Acad Sci USA*. 1999;96:7871–876.
30. Missmer SA, Hankinson SE, Spiegelman D, et al. Reproductive history and endometriosis among premenopausal women. *Obstet Gynecol*. 2004;104:965–974.
31. Missmer SA, Hankinson SE, Spiegelman D, et al. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. *Am J Epidemiol*. 2004;160:784–796.

Interaction between cytochrome P450 gene polymorphisms and serum organochlorine TEQ levels in the risk of endometriosis

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Running title: CYP gene, organochlorines and endometriosis

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Abstract

Exposure to dioxins and polychlorinated biphenyls (PCBs) has been suggested as a possible etiologic factor for endometriosis, but the association remains highly controversial. Here, to assess whether cytochrome P450 (CYP) gene polymorphisms modulate the effect of dioxins and/or PCBs in endometriosis risk, we conducted a case-control study among infertile Japanese women. A total of 138 eligible women aged 20-45 years old were diagnosed laparoscopically and classified into three subgroups: control (no endometriosis), early endometriosis (stage I-II) and advanced endometriosis (stage III-IV). Neither CYP1A1 Ile462Val and CYP1B1 Leu432Val polymorphisms (genotypes with vs. genotypes without the minor allele) nor serum dioxin and PCB toxic equivalency (TEQ) levels (low vs. high) were independently associated with either early or advanced endometriosis risk. However, genotypes with the CYP1A1 462Val allele showed a statistically significant reduced risk of advanced endometriosis in combination with high serum dioxin TEQ levels (adjusted odds ratio = 0.13, 95% confidence interval: 0.02-0.76) (P for interaction = 0.08). Although no association was found between serum PCB TEQ level and advanced endometriosis in any stratum of CYP1B1 Leu432Val polymorphism, a statistically significant interaction was found (P for interaction = 0.05). The CYP1A1 and CYP1B1 polymorphisms may modify the relation between environmental exposure to organochlorine and advanced endometriosis risk.

Keywords: CYP1A1, CYP1B1, endometriosis, gene-environment interaction, organochlorine

Introduction

Exposure to certain xenoestrogens, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCBs) has been suggested as a possible etiologic factor for endometriosis. The association between endometriosis and these organochlorines has been the subject of a number of studies (Mayani *et al.*, 1997; Lebel *et al.*, 1998; Pauwels *et al.*, 2001; Eskenazi *et al.*, 2002; Heilier *et al.*, 2005; Louis *et al.*, 2005; Tsukino *et al.*, 2005), but remains highly controversial. At this time, there is insufficient evidence to establish a definitive link between endometriosis and organochlorine exposure.

Endometriosis, an estrogen-dependent disease, is regarded as a complex trait influenced by both genetic and environmental factors (Kennedy, 1998). To understand this condition, consideration must be given to both the individual contributions of genetic and environmental factors and their magnitude, and also the interactions of these factors. Gene-environment interactions, the multiplicative joint effects of genetic predisposition and environmental factors, are important in understanding how risk factors act together and in identifying high-risk groups (Brennan, 2002).

Genetic polymorphisms in cytochrome P450 (CYP) 1A1 and CYP1B1 are putative genetic factors associated with inter-individual susceptibility to organochlorines. CYP1A1 and CYP1B1 are phase I drug-metabolizing enzymes that are critical to both xenobiotic and estrogen metabolism. The activities of CYP1A1 and CYP1B1 are determined jointly by genetic and environmental factors (Gonzalez, 1988; Martucci and Fishman, 1993). Inconsistent associations between endometriosis and organochlorine exposure might be attributable to the different genetic susceptibilities in the populations studied.

The magnitude of risk associated with gene-environment interactions can be estimated

from a case-control study. In the present study, we tested the hypothesis that the genetic polymorphisms CYP1A1 Ile462Val and CYP1B1 Leu432Val modulate the effect of dioxins and/or PCBs in the risk of endometriosis, and thereby assessed the possibility that altered risk arises from genetic predisposition.

Materials and Methods

Study population

This study was part of a case-control study conducted on a Japanese population to investigate associations between genetic and environmental factors in endometriosis (Tsukino *et al.*, 2005). Consecutive female patients aged 20- to 45-years-old who attended the Department of Obstetrics and Gynecology at Jikei University School of Medicine Hospital for infertility between 1999 and 2000 were recruited. Since pregnancy commonly results in complete resolution of minimal or mild endometriosis, women who had given birth or lactated were ineligible, leaving a total of 159 women who met the criteria. After excluding 15 women who did not give consent, 5 who did not undergo blood screening or laparoscopic examination, and 1 whose DNA sample was not available, a total of 138 women were available for the study (participation rate = 87%). No participants had undergone prior empiric therapy before laparoscopic examination.

All study protocols were approved by the Institutional Review Boards of Jikei University, National Cancer Center, University of Miyazaki, National Institute for Environmental Studies and the U.S. Centers for Disease Control and Prevention (CDC). All participants provided written informed consent before laparoscopic examination.

Before the laparoscopic examination, participants were interviewed by a single trained

interviewer using a structured questionnaire. Participants also gave a fasting blood sample before the laparoscopic examination. Blood samples were divided into plasma and buffy layers and stored at −80 °C until analysis.

Diagnosis of endometriosis

In the present study, all participants underwent diagnostic laparoscopy as part of an infertility work-up. Laparoscopy is essential to the accurate diagnosis of endometriosis because one-third of women with endometriosis are asymptomatic (Rawson, 1991). The degree of endometriosis was diagnosed according to the Revised American Fertility Society (r-AFS) classification (American Fertility Society, 1985) and/or histologic diagnosis. Endometriosis was absent in 59 women (43%), stage I in 21 women (15%), stage II in 10 women (7%), stage III in 23 women (17%) and stage IV in 25 women (18%). Current theories of endometriosis suggest that what is defined as minimal/mild endometriosis may actually represent a normal physiologic process. Furthermore, a lack of consistency between laparoscopic and histologic diagnosis has been reported, particularly for minimal/mild endometriosis (Marchino *et al.*, 2005). Considering the more severe stages as a separate category thus appears reasonable (Zondervan *et al.*, 2002). Although women without endometriosis and with stage I were designated as controls and women with stage II or more severe endometriosis were designated as cases in the previous study (Tsukino *et al.*, 2005), considering the current theories of endometriosis mentioned above, we classified cases into two subgroups in the present study: early (stage I-II) or advanced endometriosis (stage III-IV). Women without endometriosis were defined as controls. Among controls, several conditions known to cause infertility were confirmed laparoscopically, including