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The *Asp*^{327}*Asn* polymorphism in the sex hormone-binding globulin gene modifies the association of soy food and tea intake with endometrial cancer risk

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Abstract

We evaluated the interactive effect of polymorphisms in the sex hormone-binding globulin *(SHBG)* gene with soy isoflavones, tea consumption and dietary fiber on endometrial cancer risk in a population-based case-control study of 1,199 endometrial cancer patients and 1,212 controls. Genotyping of polymorphisms was performed by using TaqMan assays (rs6259) or the Affymetrix MegAllele Targeted Genotyping System (rs13894, rs858521 and rs2955617). Dietary information was obtained using a validated food frequency questionnaire. A logistic regression model was employed to compute adjusted odds ratios (ORs) and 95% confidence intervals (CIs). We found that the *Asp*^{327}*Asn* (rs6259) polymorphism was associated with decreased risk of endometrial cancer, particularly among post-menopausal women (OR =0.79, 95%CI: 0.62-1.00). This single nucleotide polymorphism (SNP) modified associations of soy isoflavones and tea consumption but not fiber intake with endometrial cancer, with the inverse association of soy intake and tea consumption being more evident for those with the *Asp/Asp* genotype of the *SHBG* gene at *Asp*^{327}*Asn* (rs6259), particularly pre-menopausal women ($P_{interaction}$ = 0.06 and 0.02, respectively, for soy isoflavones and tea intake). This study suggests that gene-diet interaction may play an important role in the etiology of endometrial cancer risk.

Keywords

Soy food; tea; polymorphism; endometrial cancer; sex hormone binding globulin

Introduction

Sex steroid hormones play a central role in the development of endometrial cancer. It has been suggested that sex hormone-binding globulins (SHBG) modulate the bioavailability of sex hormones to the target tissues by binding with the circulating sex hormones (1,2). SHBG also functions as an active regulator of the steroid-signaling system in target cells (3,4). Several

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epidemiologic studies have consistently shown that high blood levels of SHBG are associated with reduced endometrial cancer risk in post-menopausal women (5,6).

Production and clearance of SHBG are influenced by many stimulatory and inhibitory factors. For example, a functional genetic variant in the *SHBG* gene, *Asp*^{327}*Asn (rs6259)*, has been shown to result in a decrease in the clearance rate of SHBG, an increase in the half-life of the protein (7), and an elevated blood level of SHBG (7-11), particularly among post-menopausal women (9-11). Dietary fiber has been observed to affect circulating levels of SHBG (12), although the results are not consistent (13). Soy foods and their constituents, isoflavones, may also stimulate the production of SHBG (14-16).

A recent report showed a significant interactive effect of isoflavone intake and *SHBG* genetic polymorphisms on circulating SHBG levels (17). In our previous studies, we also found that soy food intake and tea consumption interact with several estrogen-related genes such as *UGT1*, *HSD17β1* and *CYP19A1* in endometrial cancer (18-20). We hypothesized that these dietary factors may interact with *SHBG* polymorphisms in the development of endometrial cancer and tested this hypothesis in the Shanghai Endometrial Cancer Study (SECS), a population-based case-control study conducted in Shanghai, China.

Materials and Methods

Study Subjects

Details of the SECS have been described elsewhere (21,22). Briefly, this study included 1,199 incident endometrial cancer cases diagnosed between 30 and 69 years of age from 1997 to 2003 and 1,212 age-frequency matched community controls. Through the population-based Shanghai Cancer Registry, 1,449 eligible endometrial cancer cases were identified during the study period, of which 1,204 cases (82.7%) completed in-person interviews.

Controls were randomly selected from the general population of Shanghai using the Shanghai Resident Registry and were matched to cases according to the age distribution of endometrial cancer cases in 1996. Women with a history of cancer or hysterectomy were not eligible. Of the 1,629 eligible women contacted, 1,212 (74.4%) participated in the study. The study protocols were approved by the Institutional Review Boards of all institutes involved in the study, and written, informed consent was obtained from all participants prior to participation in the study.

Study participants were interviewed in person by trained retired medical professionals. A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. Tea drinkers were defined as women drinking tea at least three times per week for 6 months or longer. Anthropometrics were also taken at the time of the interview.

Information on usual dietary intake during the five years preceding the interview was collected using a validated quantitative food-frequency questionnaire that covers more than 85% of foods commonly consumed in Shanghai (23). Specific nutrient intakes, including soy isoflavones and dietary fiber intake, were estimated by using the nutrient content listed in the Chinese Food Composition Tables (24).

SNP selection, identification and genotyping

Haplotype-tagging SNPs (htSNPs) were chosen using the pairwise tagging approach (25). This method (Tagger, Paul de Bakker, http://www.broad.mit.edu/mpg/tagger/) has been implemented in the HapMap project (http://www.hapmap.org). We used HapMap Han Chinese

(HCB) data for the htSNPs search. The htSNPs selection criteria were: the SHBG gene and the 5kb upstream and downstream regions, the r^2 cut off = 0.9, and a MAF ≥ 0.05. A total of four htSNPs (rs13894, rs858521, rs6259 and rs2955617) were identified for genotyping. The last time to access HapMap for htSNPs selection was December 21, 2005.

Of the study participants who completed an in-person interview, 850 cases and 853 controls donated a blood sample and 280 cases and 274 controls provided a buccal cell sample (187 cases and 186 controls provided samples using a mouthwash method; and 93 cases and 88 controls provided samples using a buccal swab method). Due to the very low DNA yield of the buccal swab method, we did not include buccal swab DNA samples in the genotyping. In addition, DNA samples from 19 control subjects who donated a blood sample were used up in other studies. Thus, DNA samples from 1,037 cases (86.5%, 850 blood and 187 buccal cell) and 1,020 controls (84.2%, 834 blood and 186 buccal cell) were included in the genotyping study. *SHBG* genotyping data were obtained from 1,028 cases and 1,016 controls, a success rate of 99.1% and 99.6%, respectively.

Genomic DNA was extracted from buffy coat fractions or buccal cells using a QIAamp DNA mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Genotyping for rs6259 (*Asp*^{327}*Asn*) was conducted using the TaqMan genotyping assay (Assay ID: 11955739_10, Applied Biosystems, Foster City, CA) in ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems) as described previously (22). SNPs rs13894, rs858521, and rs2955617 were genotyped with Affymetrix MegAllele Targeted Genotyping System by using a Molecular Inversion Probe (MIP) method (26) at the Vanderbilt Microarray Shared Resource following the manufacturer's protocol, as a part of large genotyping effort including 1,737 SNPs. Briefly, 2.01 ug of genomic DNA was annealed to the assay panel overnight at 58°C. Following annealing, the samples were split into 4 equal aliquots. Each aliquot was gap filled with each of the 4 different aliquots receiving a different dNTP. The dNTP was ligated to produce a padlocked probe and then digested with exonucleases. The padlocked probe was then cleaved at a specific cleavage site and inverted. This inverted probe was the substrate for two rounds of PCR. After passing quality control, the samples were hybridized. Following hybridization, the arrays were washed, stained, detected via the scanner, and analyzed by Affymetrix protocol.

The laboratory staff was blind to the identity of the subjects. Quality control (QC) samples were included in the genotyping assays. For SNP rs6259 genotying, each 384-well plate contained four water, eight CEPH 1347-02 DNA, eight blinded QC samples, and eight unblinded QC samples. The blinded and unblinded QC samples were taken from the second tube of study samples included in the study. The agreement of the genotypes determined was 98.7% among the duplicate samples. In addition, we genotyped 45 DNA samples from the Chinese participants used in the International HapMap project as an additional quality control. The genotypes of the samples generated from our study were compared to data downloaded from HapMap (http://www.hapmap.org). The concordance rates between the data generated in our lab and the data from the HapMap database was 100%. We included 39 blinded QC samples and 12 HapMap DNA samples in the Affymetrix MegAllele Targeted Genotyping System as a QC procedure. The average consistency rates were 99.6% for both QC samples and HapMap DNA samples.

Statistical Analyses

Chi-squared statistics were used to evaluate case-control differences in the distribution of genotypes. Haplotypes for the four SNPs were constructed based on their chromosome position (rs13894-rs858521-rs6259-rs2955617) via a Bayesian approach using PHASE software (27, 28). Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (95%CIs) with adjustment for potential confounding variables. Covariates adjusted

for included age (continuous variable), education (no formal education/elementary/middle school/high school/college), menopausal status (pre-/post-menopausal), years of menstruation (<25, <30, <35, ≥35 yrs), number of pregnancies (0, 1, 2, 3, 4, ≥5), diagnosis of diabetes (ever/never), body mass index (by quintile), alcohol consumption (never/ever), oral contraceptive use (ever/never), physical activity in metabolic equivalent tasks (METs) (by quintile), and total energy intake (by quintile). Dietary polyphenol-cancer risk associations did not change substantially by additionally adjusting for total fruit and vegetable intake, thus these results were not presented in the tables. Interactions of dietary factors with *SHBG* polymorphisms were evaluated in logistic regression analyses using the likelihood ratio test by comparing the model including the main effects only with that including both the main effects and the interaction terms. All statistical tests were based on two-tailed probability.

Results

The distribution of alleles of *SHBG* polymorphisms among cases and controls is summarized in Table 1. All four SNPs were consistent with Hardy-Weinberg equilibrium among controls (P >0.05). Slightly more cases carried the rs6259 *Asp/Asp* genotype than did controls (71.1% and 68.0% for cases and controls, respectively; P=0.07). Women with the *Asp/Asn* or *Asn/Asn* genotype at rs6259 had a slightly lower risk of endometrial cancer compared to women with the *Asp/Asp* genotype ($OR_{age\text{-}adjusted}$ =0.86, 95%CI, 0.72-1.04). Genotype frequencies of rs13894, rs858521 and rs2955617 were similar among cases and controls, and no significant associations were observed for these polymorphisms with the risk of endometrial cancer.

Based on observed genotyping data, the estimated common haplotype (≥3%) frequencies for the SNPs are also shown in Table 1. The estimated frequency of the *SHBG* haplotypes was not significantly different between cases and controls ($P = 0.45$). Compared with the most common haplotype, an non-significant inverse association was observed for other major haplotypes, particularly for the haplotype CCAG, the only common haplotype containing the variant allele of SNP rs6259 (as presented in Table 1).

The association between *SHBG* polymorphisms and endometrial cancer risk stratified by menopausal status are presented in Table 2. The inverse association between SNP rs6259 and endometrial cancer appeared to be confined to post-menopausal women ($OR_{age\text{-}adjusted}$= 0.79; 95% CI, 0.62-1.00 for post-menopausal women and $OR_{age\text{-}adjusted}$ =1.02; 95% CI, 0.75-1.37 for pre-menopausal women), although the P value for the interaction test was not significant (P=0.19). Similarly, haplotype CCAG was associated with a 18% decreased risk of endometrial cancer compared with the CCGG haplotype among post-menopausal women (95%CI, 0.65-1.03). Haplotype TCGG, on the other hand, was related to lower risk of endometrial cancer among pre-menopausal women (OR=0.63, 95%CI, 0.39-1.01) compared with the CCGG haplotype.

The potential joint effects of *SHBG* genotype and dietary factors on endometrial cancer risk are evaluated in Table 3 and Table 4. Dietary fiber was associated with a slightly lower risk of endometrial cancer regardless of *SHBG* genotype. The inverse associations of soy isoflavones and tea consumption with endometrial cancer were more evident among women with the *Asp/Asp* genotype (Table 3). These association patterns appeared to be more pronounced among pre-menopausal women (Table 4). Among pre-menopausal women, the adjusted OR for women with the *Asp/Asp* genotype was 0.48 (95% CI: 0.30-0.78) for the highest tertile intake of soy isoflavones (P_{trend}<0.01), while the corresponding OR was 0.95 (95%CI: 0.50-1.79) for *Asn* allele carriers (P_{trend} =0.50) compared with women with the *Asp/Asp* genotype who were in the lowest tertile of intake. Similarly, the protective effect of tea consumption on endometrial cancer was more pronounced among pre-menopausal women with the *Asp/Asp* genotype ($P_{interaction}$=0.02).

Discussion

In this large scale case-control study, we found that the *SHBG* rs6259 polymorphism was associated with the risk of endometrial cancer among post-menopausal women and modified diet-endometrial cancer associations among pre-menopausal women.

Endometrial cancer is a sex hormone-related disease. Sex hormone-binding globulin (SHBG) plays a role in endometrial carcinogenesis, possibly by modulating the bioavailability of circulating sex hormones (1,2). Increased production of SHBG, caused either by genetic variations in the *SHBG* gene or dietary factors, may result in an increase in the levels of inactive, bound sex hormones and a decrease in the concentration of active, unbound or free hormones. Higher levels of SHBG have been associated with lower post-menopausal endometrial cancer risk (5,6).

SHBG is coded by the *SHBG* gene, which is located at chromosome 17p12-p13 (29). Several genetic variations in the *SHBG* gene, such as a common missense single nucleotide polymorphism in exon 8 (*Asp*^{327}*Asn*, rs6259) and a functional pentanucleotide repeat polymorphism (TAAAA)n in the 5′promoter region, have been shown to alter circulating levels of SHBG and influence the pathogenesis of estrogen-related cancers (9). In our Asian study population, we evaluated four haplotype tagging SNPs in the *SHBG* gene chosen based on a minor allele frequency ≥ 0.05 and $r^2 \geq 0.90$. We found that the rs6259 polymorphism was associated with a reduced risk of endometrial cancer among post-menopausal women. Our finding is biologically plausible. The *Asp*^{327}*Asn* polymorphism is a nonsynonymous SNP (G to A) at nucleotide 5790 in exon 8 of the *SHBG* gene, which results in an amino acid substitution of asparagine for aspartic acid at residue 327 (*Asp*^{327}*Asn*, rs6259) in the SHBG polypeptide. This change generates an additional N-linked carbohydrate chain attached to the SHBG molecule, resulting in a decrease in the clearance rate of this protein. In our previous reports, we found that the ^{327}*Asn* variant was associated with 12% higher plasma levels of SHBG (11), and a reduced risk of endometrial cancer (22) and breast cancer (11) only among post-menopausal women. The null association between the *Asp*^{327}*Asn* polymorphism and SHBG levels and between this polymorphism and endometrial cancer risk among pre-menopausal women may be explained by the confounding effect of high levels of estrogen on the SHBG genotype-phenotype association.

Dietary fiber intake has been linked to reduced risk of endometrial cancer (30), possibly through modification of SHBG levels (12,13). In this study, we observed a weak inverse association between dietary fiber intake and cancer risk among both women with the Asp/Asp genotype and women who were Asn carriers. On the other hand, the protective effect of tea consumption on endometrial cancer was observed only among women with the Asp/Asp genotype, particularly among pre-menopausal women. We previously reported an interaction of tea consumption with the *CYP19A1* gene and endometrial cancer risk, which may be attributable to the inhibitory effects of tea polyphenols on aromatase activity (20). Although the mechanism underlying the modifying effect of tea on the *SHBG* gene-endometrial cancer association is unclear, such diet-gene interactions could be due to the effect of tea polyphenols on estrogen metabolism. Further studies are warranted on this issue.

Intake of isoflavones has been consistently shown to increase SHBG levels in post-menopausal women (31-33), including in an intervention study (31). However, it has also been suggested that intake of soy protein supplements, with and without isoflavones, decreases concentrations of SHBG in post-menopausal women (34). In a recent report from a study of post-menopausal European women, isoflavones were found to increase SHBG levels in a dose-response manner among women carrying the *Asn* variant, suggesting that the effect of isoflavones on hormone-related diseases may be modified by SHBG (17). In the current study, we found that the

Asp^{327}*Asn* polymorphism modified the effect of soy food on endometrial cancer risk. The soy food effect was predominantly seen among pre-menopausal women who carried the *Asp/Asp* genotype, a group of women who presumably have a high level of estrogen exposure. Soy food consumption was inversely, but much more weakly, associated with endometrial cancer risk among post-menopausal Chinese women, and this association was not modified by the *Asp*^{327}*Asn* polymorphism. Our findings appear to be contradictory to the findings of the European study (17) but are biologically plausible. Isoflavones have both anti-estrogenic and estrogen-like effects, depending on the endogenous estrogen level (35). Among pre-menopausal women, particularly those with the *Asp/Asp* genotype, endogenous estrogen levels are high. In this group of women, soy isoflavones may act as an estrogen antagonist and reduce the risk of endometrial cancer. Conversely, among ^{327}*Asn* carriers or post-menopausal women, biologically available estrogen levels are low, and isoflavones may therefore have little anti-estrogenic effect or may even have an estrogen-like effect. More studies are needed to test this hypothesis and verify our findings.

To our knowledge, this is the first study to evaluate diet-*SHBG* gene interaction with endometrial cancer risk in a large, population-based case-control study. The strength of the study includes the population-based design, the relatively high participation rate (82.7% for cases and 74.4% for controls), high DNA sample donation rates, and the low frequency of hysterectomy in the study population. The relatively homogeneous ethnic background (>98% Han Chinese) of our population also decreases the potential confounding effect of ethnicity for genotyping data, and the application of the haplotype tagging SNP approach in SNP selection made it possible to capture all potentially functional markers common in the *SHBG* gene.

As with all case-control studies the potential for recall bias could not be eliminated. Because neither study participants nor interviewers were aware of our diet and endometrial cancer hypothesis, any misclassification is likely to be non-differential and result in an underestimation of the diet-disease association. Finally, given that multiple genes are involved in estrogen biosynthesis and metabolism (9,36), the confounding and/or modifying effects of other genes also cannot be excluded.

In summary, we found that the *SHBG* rs6259 polymorphism influences the risk of endometrial cancer, and the reduced risk is dependent on endogenous hormone levels and interacts with dietary polyphenol intake.

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Table 1

Genotype and haplotype frequencies of the *SHBG* gene and associations with endometrial cancer, the Shanghai Endometrial Cancer Study, 1997-2003.

Genotypes	**Location**	**Position**	**Cases**	**Controls**	***P for χ^2 test***	**Age adjusted ORs (95% CI)**	***P for trend***
rs13894	flanking	Chr. 17	1028	1001			
C/C		7470627	968	936	*0.54*	1.00	
C/T			60	65		0.89(0.62-1.28)	
T/T			0	0			
P for HWE [a]			*0.34*	*0.29*			
rs858521	boundary	Chr. 17	1027	1003			
C/C		7470872	554	547	*0.96*	1.00	
C/G			420	397		1.04(0.87-1.25)	
G/G			53	59		0.89(0.60-1.31)	*0.96*
C/G, G/G			473	456	*0.79*	1.02(0.86-1.22)	
P for HWE [a]			*0.02*	*0.24*			
rs6259	exon 8	Chr. 17	1028	1016			
G/G		7477252	731	691	*0.07*	1.00	
G/A			283	302		0.89(0.73-1.07)	
A/A			14	23		0.58(0.29-1.13)	*0.07*
G/A, A/A			*297*	*325*	*0.13*	0.86(0.72-1.04)	
P for HWE [a]			*0.02*	*0.13*			
rs2955617	3′UTR	Chr. 17	1025	1002			
G/G		7479510	311	317	*0.65*	1.00	
G/T			528	504		1.07(0.88-1.30)	
T/T			186	181		1.05(0.81-1.35)	*0.66*
G/T, T/T			714	685	*0.53*	1.06(0.88-1.28)	
P for HWE [a]			*0.14*	*0.43*			

Haplotypes [b]	Frequency in cases	Frequency in controls	Cases	Controls	*P for χ^2 test*	ORs (95%CI) [c]
CCGG	37.9	36.0	833	811		1.00
CGGT	25.3	25.3	557	574		0.95(0.81-1.10)
CCGT	18.4	17.9	415	421		0.96(0.81-1.13)
CCAG	15.2	17.1	338	368		0.89(0.75-1.07)
TCGG	2.9	3.2	78	103		0.74(0.54-1.01)
Others	0.3	0.4	19	23	0.37	0.80(0.44-1.49)
P for 100 times permutation test=0.45						

[a] Hardy-Weinberg Equilibrium.

[b] In the order SNP rs13894, rs858521, rs6259 and rs2955617 based on their chromosome position.

[c] Not adjusted for any variables.

Table 2

Association of *SHBG* genotypes and haplotypes with the risk of endometrial cancer by menopausal status, the Shanghai Endometrial Cancer Study, 1997-2003.

	Pre-menopausal women		Post-menopausal women		*P for interaction*
***SHBG* genotypes**	**Cases/Controls**	**OR (95%CI)**	**Cases/Controls**	**OR (95%CI)**	
rs13894					
C/C	418/356	1.00 [a]	550/580	1.00 [a]	
C/T	26/29	0.76(0.44-1.32)	34/36	1.02(0.63-1.65)	*0.49*
rs858521					
C/C	237/203	1.00 [a]	317/344	1.00 [a]	
C/G, G/G	207/183	0.97(0.73-1.27)	266/273	1.06(0.84-1.33)	*0.60*
rs6259					
G/G	314/281	1.00 [a]	417/410	1.00 [a]	
G/A, A/A	127/113	1.02(0.75-1.37)	170/212	0.79(0.62-1.00)	*0.19*
rs2955617					
G/G	131/120	1.00 [a]	180/197	1.00 [a]	
G/T	232/190	1.11(0.81-1.53)	296/314	1.04(0.80-1.34)	
T/T	81/76	0.98(0.66-1.47)	105/105	1.10(0.78-1.54)	*0.44*
	P for trend	*0.96*		*0.60*	
Haplotypes [b]					
CCGG	350/316	1.00 [c]	483/495	1.00 [c]	
CGGT	243/235	0.93(0.74-1.18)	314/339	0.95(0.78-1.16)	
CCGT	175/161	0.98(0.76-1.28)	240/260	0.95(0.76-1.17)	
CCAG	147/129	1.03(0.78-1.36)	191/239	0.82(0.65-1.03)	
TCGG	32/46	0.63(0.39-1.01)	46/57	0.83(0.55-1.24)	
Others	7/7	0.90(0.31-2.60)	12/16	0.77(0.36-1.64)	

[a] Age adjusted ORs.

[b] In the order SNP rs13894, rs858521, rs6259 and rs2955617 based on their chromosome position.

[c] Unadjusted ORs.

Table 3

Association of dietary factors with endometrial cancer risk by SHBG genotypes at *rs6259*, the Shanghai Endometrial Cancer Study, 1997-2003.

	All subjects		Asp/Asp genotype		Asp/Asn and Asn/Asn genotype		P for interaction
	Cases/Controls	ORs (95%CI)	Cases/Controls	ORs (95%CI)	Cases/Controls	ORs (95%CI)	
Dietary fiber intake (g/d, by tertile)							
≤9.0	394/404	1.00	237/223	1.00	93/104	1.00	0.99
9.1-12.7	417/405	0.93(0.75-1.16)	264/242	0.94(0.70-1.25)	101/106	0.95(0.59-1.52)	
>12.7	388/403	0.76(0.59-0.97)	230/226	0.79(0.57-1.10)	103/115	0.72(0.43-1.22)	
	P for trend	*0.03*		*0.16*		*0.22*	
Soy isoflavone intake (mg/d, by tertile)							
≤21.3	404/404	1.00	247/226	1.00	94/104	1.00	0.34
21.4-40.3	407/405	0.87(0.71-1.08)	263/232	0.90(0.68-1.18)	92/114	0.71(0.45-1.11)	
>40.3	388/403	0.76(0.61-0.96)	221/233	0.69(0.51-0.94)	111/107	0.88(0.55-1.42)	
	P for trend	*0.02*		*0.02*		*0.60*	
Tea consumption							
Never	842/834	1.00	527/465	1.00	192/230	1.00	0.03
Ever	357/378	0.78(0.64-0.94)	204/226	0.67(0.52-0.86)	105/95	1.19(0.80-1.76)	

Adjusted for age, education, menopausal status, years of menstruation, number of pregnancies, oral contraceptive use, alcohol consumption, diagnosis of diabetes, body mass index, physical activity and caloric intake.

Table 4

Joint effect of soy protein intake and tea consumption on *SHBG* genotypes at *rs6259*, stratified by menopausal status, the Shanghai Endometrial Cancer Study, 1997-2003.

	Pre-menopausal women with genotyping data			P for interaction	Post-menopausal women with genotyping data			P for interaction
	Cases/Controls	Asp/Asp	Asp/Asn, Asn/Asn		Cases/Controls	Asp/Asp	Asp/Asn, Asn/Asn	
Dietary fiber intake (g/d, by tertile)								
≤9.0	140/136	1.00	0.88(0.49-1.58)	0.54	190/191	1.00	0.80(0.50-1.28)	0.71
9.1-12.7	163/117	1.06(0.67-1.67)	1.43(0.79-2.59)		202/231	0.82(0.57-1.19)	0.60(0.37-0.96)	
>12.7	138/141	0.64(0.39-1.05)	0.76(0.41-1.41)		196/200	0.85(0.56-1.29)	0.56(0.34-0.92)	
	P for trend	*0.17*	*0.18*			*0.64*	*0.18*	
Soy isoflavone intake(mg/d, by tertile)								
≤21.3	155/136	1.00	1.08(0.61-1.92)	0.06	186/194	1.00	0.76(0.47-1.22)	0.97
21.4-40.3	169/133	0.93(0.61-1.43)	0.71(0.41-1.24)		187/213	0.84(0.58-1.22)	0.56(0.35-0.91)	
>40.3	117/125	0.48(0.30-0.78)	0.95(0.50-1.79)		215/215	0.83(0.56-1.22)	0.63(0.39-0.99)	
	P for trend	*<0.01*	*0.50*			*0.36*	*0.45*	
Tea consumption								
Never	286/251	1.00	0.84(0.55-1.27)	0.02	434/444	1.00	0.68(0.49-0.93)	0.52
Ever	155/143	0.65(0.44-0.94)	1.24(0.73-2.09)		154/178	0.73(0.51-1.02)	0.64(0.41-1.01)	

Adjusted for age, education, years of menstruation, number of pregnancies, oral contraceptive use, diagnosis of diabetes, alcohol consumption, body mass index, physical activity, and caloric intake.