CYP17 Genotype Modifies the Association between Lignan Supply and Premenopausal Breast Cancer Risk in Humans[1]

Regina Piller,* Emaculate Verla-Tebit,† Shan Wang-Gohrke,** Jakob Linseisen,*† and Jenny Chang-Claude†[2]

*Unit of Human Nutrition and Cancer Prevention, Technical University of Munich, Germany; †German Cancer Research Center, Department of Clinical Epidemiology, Heidelberg, Germany; and **Molecular Biology Laboratory, Department of Obstetrics and Gynecology, University of Ulm, Germany

ABSTRACT Cytochrome P450c17α (CYP17) has been associated with alterations in steroid hormone levels and premenopausal breast cancer risk and could modify the association between phytoestrogen intake and breast cancer risk. We examined plasma concentrations of enterolactone and genistein, estimated dietary phytoestrogen intake, CYP17 5′-UTR *Msp*A1 genetic polymorphism, and breast cancer risk in 267 premenopausal breast cancer patients and 573 age-matched population controls from Germany. Multivariate logistic regression was used to estimate breast cancer risk associated with quartiles of phytoestrogen intake by genotype and to investigate gene-nutrient interactions. Premenopausal breast cancer risk was not significantly associated with the CYP17 A2 genotype. We observed a significant modifying effect of CYP17 genotype on plasma enterolactone–associated breast cancer risk (P for interaction < 0.01). Plasma enterolactone was significantly inversely related to breast cancer risk only in A2A2 carriers, showing odds ratios and 95% CI of 0.02 (0.00–0.41) and 0.01 (0.00–0.21) for the third and fourth quartiles vs. the lowest quartile, respectively. This inverse association was also found for the calculated enterolignan production as well as matairesinol intake. Compared with A1A1 carriers with the lowest enterolactone supply, the risk reduction associated with a high enterolactone supply resulted in a comparably decreased breast cancer risk for all genotypes. For genistein, no clear indication for a differential effect by CYP17 genotype was obtained. Our results suggest that CYP17 genotype modifies the protective effect of lignans on premenopausal breast cancer risk. Women homozygous for A2 allele benefit most from high plasma enterolactone concentrations and a high consumption of dietary precursors.

Phytoestrogens are plant components structurally similar to estrogens, showing estrogenic as well as antiestrogenic properties (1). There are 2 main classes relevant to human nutrition. The isoflavonoids (genistein and daidzein) are mainly consumed with soy and legumes and thus do not play such an important role in Western compared with Asian diets. The lignans are more widespread in the plant kingdom as components of seeds (above all, flaxseed), nuts, berries, fruits, and cereals (2). The plant lignans (matairesinol and secoisolariciresinol) become biologically available as enterolignans (enterolactone and enterodiol) after fermentation by human gut microflora (3).

Epidemiological studies relying on dietary intake to investigate the role of soy or related phytoestrogens in breast cancer support a protective effect at high consumption levels. In Western countries with low soy intake, studies have produced less consistent results (4–7). This may be partly due to the paucity of data on phytoestrogen concentrations in food and dietary intakes. Several studies using urine and serum concentrations of phytoestrogens and their metabolites as biomarkers of their intake suggest that high phytoestrogen concentrations are associated with a reduced risk for breast cancer (8–10). Others have failed to show such an effect of phytoestrogens on breast cancer risk (11–14). Some of the differences in results could be related to other factors, such as genetic factors, which may modulate the effect of phytoestrogens. Circulating endogenous sex hormone levels are associated with increased risk for postmenopausal as well as premenopausal breast cancer (15,16). Functional polymorphisms in genes that encode for enzymes involved in the biosynthesis or metabolism of estrogens are therefore of particular interest.

The cytochrome P450c17α (CYP17) gene encodes for the cytochrome P450c17α enzyme and is mainly expressed in ovaries and the adrenal cortex. It mediates 17α-hydroxylase and 17,20-lyase activities, and catalyzes a rate-limiting step in estradiol biosynthesis (17,18). The 5′ untranslated region of CYP17 contains a single T to C nucleotide polymorphism (MspA1 polymorphism) that results in a wild type tt homozygote (A1A1), a tc heterozygote (A1A2), and the cc homozygote (A2A2). This polymorphism was hypothesized to create an

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[2] To whom correspondence should be addressed. E-mail: j.chang-claude@dkfz-heidelberg.de.

additional Sp1-type (CCACC box) promoter motif, which could lead to upregulation of gene expression and increased enzyme activity and thereby enhance the amount of bioavailable estrogen (19). In vitro studies have not confirmed this (20). However, carriers of at least one A2 allele had higher endogenous hormone levels compared with the A1A1 homozygotes in studies of premenopausal as well as postmenopausal women (21,22). There is also some evidence that the A2 allele may be associated with a small increase in breast cancer risk (23–26) and weak modifying effects, although the results are inconsistent (27–30).

Some dietary phytoestrogens are substrates for steroidogenic enzymes and can act as inhibitors of key enzymes of steroid metabolism (31–33). The CYP17 genotype was found to modify the effect of dietary lignans on premenopausal breast cancer risk in one study (34).

We previously observed a significant inverse association between premenopausal breast cancer risk and the dietary intake of different types of phytoestrogens (4). The protective effect of high lignan intake was confirmed using measurements of plasma enterolactone (ENL) concentrations (35). Therefore, we were interested in investigating whether the CYP17 genotype modifies the association between different types of phytoestrogens and premenopausal breast cancer risk using both dietary estimates and biomarkers of phytoestrogen intake.

MATERIALS AND METHODS

Study population. We used a population-based case-control study conducted from 1992 to 1995 in 38 hospitals of the 2 study regions of Feiburg and Rhein-Neckar-Odenwald in southern Germany. The study protocol was approved by the ethics committee of the University of Heidelberg and all study participants gave informed consent. Subjects eligible for participation were German speaking, under the age of 51 y, resided in the study regions, and had no previous history of breast cancer. Cases of newly diagnosed in situ or invasive breast cancer were identified by frequent monitoring of hospital admissions, surgery schedules, and pathology records. Median time between diagnosis and interview was 2 mo. Controls were selected from a random list of residents in the population registries. Cases and controls were matched by exact age and study region. Of 1020 eligible cases and 2257 eligible controls, 706 cases (70%) and 1381 controls (61%) participated. All subjects completed a self-administered questionnaire on demographic factors, anthropometric measures, and risk factors (36). Premenopausal women were defined as those who still had menstrual cycles or reported natural amenorrhea for <6 mo or more before the reference date (date of diagnosis for cases and date of completion of questionnaire for controls). Information on food consumption was obtained from participants in one study region by means of a validated 176-item self-administered food frequency questionnaire, which recorded nutritional habits in the year prior to the diagnosis of breast cancer (cases) or in the year prior to completing the questionnaire (controls). Complete dietary information was therefore available from 355 (278 premenopausal) cases and 838 (666 premenopausal) controls (37). Dietary phytoestrogen intake was calculated using a database of phytoestrogen-containing foods from previously established analytical data in the literature (4). In vitro data from Thompson et al. (38) was used to estimate the intestinally produced lignan metabolites, enterodiol and enterolactone. More detailed information was published previously (4).

Blood samples were requested from all participants. In cases, blood samples were collected after surgery or chemotherapy and close to the interview data (see above); samples were usually drawn during morning hours. Plasma ENL and genistein concentrations were measured in blood samples from all 220 premenopausal cases and from at least 1 matched premenopausal control (237 premenopausal controls) due to costs. The statistical analysis was restricted to 840 premenopausal women with complete information on nutritional habits and CYP17 genotype (comprising subgroup 1 were 267 cases and 573 controls). Plasma phytoestrogen concentrations were available in 454 of these premenopausal women (comprising subgroup 2 were 219 cases and 235 controls).

Laboratory analysis. ENL and genistein were analyzed by time-resolved fluoroimmunoassay according to the method designed by Adlercreutz et al. (39). The analysis was performed in duplicate according to instructions of the manufacturer (Labmaster), with minor modifications described elsewhere (35). Intra- and interassay coefficients of variation were reported to range from 3.1 to 6.1% and 6.1 to 8.6% (40). CV $<10\%$ were obtained with our 2 quality control samples analyzed within each batch.

Genotyping. Genomic DNA was extracted using Blood and Cell Culture DNA kits as described by the manufacturer (Qiagen GmbH). The CYP17 5′-UTR MspAI polymorphism (rs743572) was analyzed using the previously described PCR-fragment length polymorphism analyses assay (19,26). After amplification from genomic DNA, the PCR products were digested with the restriction endonuclease MspAI, subjected to electrophoresis through a 3% agarose gel, and visualized by staining the gel with ethidium bromide. The different genotypes were distinguished on the size of the digested fragments.

Statistical methods. Allele and genotype frequencies were calculated and deviation from Hardy-Weinberg equilibrium was tested using the χ^2-test. Associations between CYP17 genotype, dietary phytoestrogen intake, plasma phytoestrogen concentration, and risk of premenopausal breast cancer were evaluated by conditional logistic regression with stratification for exact age, providing odds ratios and their corresponding 95% CI (SAS, version 8.2). The distribution of dietary intake or plasma concentrations in controls was used for quartile definition. Analyses were adjusted for first-degree family history of breast cancer (no, yes), number of births (0, 1–2, $\geq$3), duration of breastfeeding (0, 0–6, 7–12, and $>$12 mo), oral contraceptive use (ever/never), age at menarche ($\leq$12, 13–14, and $\geq$15 y), BMI [classified as underweight ($<$18.5 kg/m^2), normal weight and overweight (18.5–30 kg/m^2), and obese ($\geq$30 kg/m^2)], alcohol consumption (0, 1–18, $>$18 g ethanol/d), educational level (low, middle, and high) as well as the day of time resolved fluoroimmunoassay measurement (day 1–16) and the time between surgery and blood sampling ($<$30, 30–182, 183–365, 366–721, and $>$721 d). For trend estimation, the median of the quartiles was assigned to each category and used as an ordinal variable in the regression analysis. Analyses were also performed stratified by CYP17 genotype. Interactions were measured by using multiplicative terms of an ordinal variable of CYP17 genotypes and a continuous variable of plasma or dietary phytoestrogen and evaluated by the log likelihood ratio test.

RESULTS

The distribution of age and known risk factors for breast cancer in cases and controls were comparable between the 2 subgroups and the original study population (**Table 1**). The allele frequencies of CYP17 genotype were in Hardy-Weinberg equilibrium and did not differ between cases and control subjects in either study group (Table 1).

For further statistical evaluation we considered only phytoestrogens previously found (4) to be significantly inversely associated with premenopausal breast cancer risk in this study population (**Table 2**). The median intake of plant lignans and genistein and the calculated intestinal production of enterolignans are similar to those found in the original study population ($\leq$1% difference). Median plasma concentrations of ENL (9.7 nmol/L) and genistein (3.7 nmol/L) are the same in the controls of both the subgroups and the original study population.

There was no significant association between CYP17 genotype and risk of premenopausal breast cancer in either subgroup (**Table 3**); however, point estimates increased with increasing number of A2 alleles.

TABLE 1

Sociodemographic variables and risk factors for breast cancer in 2 subgroups and in the original Rhein-Neckar-Odenwald study group[1]

	Subgroup 1					Subgroup 2					Original Rhein-Neckar-Odenwald study group				
	Cases, *n* = 267	%	Controls, *n* = 573	%	P^2	Cases, *n* = 219	%	Controls, *n* = 235	%	P^2	Cases, *n* = 355	%	Controls, *n* = 838	%	P^2
Age diagnosis/recruitment, *y*															
24–29	8	3.0	20	3.5	0.96	6	2.7	8	3.4	0.63	10	2.8	26	3.1	0.64
30–34	23	8.6	54	9.4		17	7.8	22	9.4		24	6.8	62	7.4	
35–39	49	18.4	105	18.3		34	15.5	45	19.1		54	15.2	130	15.5	
40–44	90	33.7	186	32.5		75	34.2	78	33.2		116	32.7	249	29.7	
45–49	83	31.1	185	32.3		76	34.7	76	32.3		128	36.1	293	35.0	
50–52	14	5.2	23	4.0		11	5.0	6	2.6		23	6.5	78	9.3	
Education level[3]															
Low	30	11.2	58	10.1	0.53	21	9.6	29	12.3	0.32	42	11.8	101	12.1	0.31
Middle	168	62.9	346	60.4		147	67.1	142	60.4		232	65.4	512	61.1	
High	69	25.8	169	29.5		51	23.3	64	27.2		81	22.8	225	26.8	
First-degree family history of breast cancer															
no	241	90.3	550	96.0	<0.01	191	87.2	227	96.6	<0.01	318	89.6	804	95.9	<0.01
yes	26	9.7	23	4.0		28	12.8	8	3.4		37	10.4	34	4.1	
Body mass index, *kg/m²*															
<18.5	8	3.0	13	2.3	0.36	8	3.7	6	2.6	0.24	12	3.4	18	2.1	0.41
18.5 to <25	173	65.0	389	67.9		140	64.2	162	68.9		229	64.7	539	64.3	
25 to <30	57	21.4	127	22.2		48	22.0	53	22.6		78	22.0	211	25.2	
30+	28	10.5	44	7.7		22	10.1	14	6.0		35	9.9	70	8.4	
Age at menarche, *y*															
≤12	105	39.3	220	38.4	0.93	79	36.1	88	37.4	0.52	145	41.0	311	37.2	0.46
13–14	128	47.9	276	48.2		109	49.8	107	45.5		163	46.0	407	48.6	
≥15	33	12.4	76	13.3		30	13.7	40	17.0		46	13.0	119	14.2	
Number of births															
0	67	25.1	131	22.9	<0.01	46	21.0	52	22.1	0.06	79	22.3	183	21.8	0.02
1 or 2	183	68.5	360	62.8		158	72.1	152	64.7		248	69.9	542	64.7	
>2	17	6.4	82	14.3		15	6.8	31	13.2		28	7.9	113	13.5	
Duration of breast feeding, *mo*															
0	122	45.7	252	44.0	0.21	98	44.7	107	45.5	0.54	167	47	378	45.1	0.09
1–6	97	36.3	182	31.8		85	38.8	79	33.6		133	37.5	286	34.1	
7–12	29	10.9	84	14.7		23	10.5	33	14.0		35	9.9	104	12.4	
≥13	19	7.1	55	9.6		13	5.9	16	6.8		20	5.6	70	8.4	
Use of oral contraceptives															
Never	48	18.0	115	20.1	0.47	36	16.4	48	20.4	0.27	60	16.9	166	19.9	0.23
Ever	219	82.0	457	79.8		183	83.6	187	19.6		295	83.1	670	80.1	
Alcohol consumption, *g/d*															
0	51	19.1	86	15.0	0.13	46	21.0	35	14.9	0.17	71	20.0	129	15.4	<0.0
1–18	182	68.2	429	74.9		149	68.0	178	75.7		235	66.2	627	74.8	1
≥19	34	12.7	58	10.1		24	11.0	22	9.4		49	13.8	82	9.8	
CYP 17 genotype[4]															
A1A1	88	33.0	209	36.5	0.57	68	31.1	84	35.7	0.53	112	32.8	257	35.4	0.71
A1A2	126	47.2	262	45.7		107	48.9	110	46.8		163	47.8	334	46.1	
A2A2	53	19.9	102	17.8		44	20.1	41	17.4		66	19.4	134	18.5	

[1] Subgroup 1 included subjects with dietary information as well as CYP17 genotype ($n = 840$); subgroup 2 included subjects with dietary information, plasma phytoestrogens concentrations, and CYP17 genotype ($n = 454$); and the original study group from the Rhein-Neckar-Odenwald region included subjects with dietary information ($n = 1193$).

[2] *P*-values represent differences between cases and controls using chi-square tests.

[3] Education levels were determined according to the type of schooling or vocational training completed: low, ≤9 years school and no training; middle, 9–10 years school and vocational or technical training; and high, ≥13 years school and vocational or technical training or university.

[4] 113 missing values in controls and 14 missing values in cases of the whole study group.

Stratified by CYP17 genotype, we observed an inverse association between plasma ENL concentrations and breast cancer risk, which was particularly strong for A2A2 carriers. Compared with those in the lowest quartile, women in the third and fourth quartile of plasma ENL had significantly reduced adjusted odds ratio (95% CI) of 0.02 (0–0.41) and 0.01 (0–0.21), respectively ($P_{trend} = 0.0015$) (**Table 4**). For both the A1A1 and A1A2 genotypes, the decrease in risk with increasing plasma ENL concentrations was not significant (*P* for interaction < 0.01). The results, based on the estimated amount of intestinally formed ENL, corresponded to those obtained for plasma ENL (**Table 5**); a significant risk reduction was also found for A2A2 carriers only. Similar results were obtained for matairesinol, the direct precursor of ENL in plants, as well as for enterodiol (Table 5). However, none of the interaction terms for the dietary phytoestrogen intake or calculated enterolignan production and CYP17 were significant at $P < 0.10$.

If only participants who gave their blood sample within 1 y after diagnosis (153 cases) or within 1 y after questionnaire completion (232 controls) were considered, the protective effect for breast cancer with increasing plasma ENL concentrations

TABLE 2

Phytoestrogen intake data and plasma concentrations in premenopausal breast cancer cases and controls

Phytoestrogens	Cases			Controls		
	Median	25th Percentile	75th Percentile	Median	25th Percentile	75th Percentile
			μg/d			
Subgroup 1[1]						
Enterolactone (calculated)	316.7	227.4	445.2	331.8	223.3	452.8
Enterodiol (calculated)	364.6	234.7	553.0	382.0	241.6	577.5
Matairesinol intake	28.0	19.6	37.7	28.8	20.5	38.6
Genistein intake	44.0	28.2	69.6	48.1	31.2	81.1
			nmol/L			
Subgroup 2[2]						
Plasma enterolactone	6.3	2.4	14.2	9.7	3.3	17.0
Plasma genistein	4.5	2.2	12.1	3.7	2.1	10.0

[1] Subgroup 1 included subjects with dietary information and CYP17 genotype; cases, $n = 267$, controls, $n = 573$ for each entry.

[2] Subgroup 2 included subjects with dietary information, plasma phytoestrogen data, and CYP17 genotype; cases, $n = 219$, controls, $n = 235$ for each entry.

became slightly stronger. There were only minor differences in results when considering dietary intake data only from subjects who completed the food frequency questionnaire within 1 y of surgery (204 cases) or recruitment (569 controls).

To estimate the joint effects of CYP17 and phytoestrogens, we used subjects carrying the A1A1 genotype with the lowest ENL supply (plasma concentrations or calculated intestinal production) as the reference group (**Fig. 1**). We observed a significantly elevated odds ratio (95% CI) of 3.6 (1.1–12.1) for A2A2 carriers with low ENL supply. However, the reduction in risk with increasing phytoestrogen concentrations was most pronounced among A2A2 carriers, yielding odds ratios (95% CI) of 0.6 (0.2–2.1) and 0.3 (0.1–1.2) for breast cancer in the third and fourth quartile of plasma ENL, respectively. Both the analyses, using the plasma ENL concentrations and the estimated intestinal production of ENL from dietary precursors, showed that homozygous carriers of the A2 allele can benefit most from a high ENL supply.

We found no clear indication for a modifying effect of CYP17 genotype on breast cancer risk associated with genistein intake or plasma concentrations (P for interaction > 0.1).

DISCUSSION

Our data suggest that the risk-reducing effect of lignan consumption on premenopausal breast cancer can be modified by CYP17 genotype. Women with the presumably high-risk genotype A2A2 benefit most from a high consumption of ENL precursors and the synthesis of their metabolites in the gut or, considering also absorption, from a high circulating ENL concentration. The concentration of metabolites in plasma is dependent upon the intake of precursors and the individual composition of the intestinal microflora (41). Therefore, the consistent results regarding matairesinol (a direct precursor of ENL), the calculated amount of intestinally produced

TABLE 3

Odds ratios (OR) and 95% CI for the association of CYP17 genotype and premenopausal breast cancer risk, by subgroups of available additional information

CYP17 Genotype	Subgroup 1[1]			Subgroup 2[2]		
	A1A1	A1A2	A2A2	A1A1	A1A2	A2A2
Cases/controls	88/209	126/262	53/102	68/84	107/110	44/41
OR, crude	1.00 (Ref.)	1.15	1.22	1.00 (Ref.)	1.29	1.32
95% CI		0.82–1.60	0.80–1.85		0.83–1.98	0.76–2.30
OR, adjusted	1.00* (Ref.)	1.13*	1.17*	1.00** (Ref.)	1.28**	1.35**
95% CI		0.80–1.59	0.76–1.82		0.81–2.01	0.75–2.43

[1] Subgroup 1 includes subjects with information on diet and CYP17 genotype, $n = 840$.

[2] Subgroup 2 includes subjects with information on plasma phytoestrogens and CYP17 genotype, $n = 454$.

* Adjusted for age at menarche, use of oral contraceptives, family history of breast cancer, number of births, duration of breastfeeding, education, alcohol consumption, BMI, deciles of total daily energy intake.

** Adjusted for age at menarche, use of oral contraceptives, family history of breast cancer, number of births, duration of breastfeeding, education, alcohol consumption, BMI.

Ref., reference group.

TABLE 4

Crude and adjusted OR and 95% CI for the risk of premenopausal breast cancer by plasma concentrations of enterolactone and genistein and CYP17 genotype (n = 454)

	Quartiles, crude results					Quartiles, adjusted results[1]				
	1	2	3	4	*P* for trend	1	2	3	4	*P* for trend
Plasma enterolactone										
median, *nmol/L*	1.37	5.96	12.96	24.96						
CYP17 A1A1										
Cases/controls	17/19	23/22	17/20	11/23						
OR	1.00	0.96	0.86	0.63	0.341	1.00	1.04	0.67	0.52	0.2050
95% CI	(Ref.)	0.38–2.45	0.33–2.23	0.23–1.74		(Ref.)	0.35–3.06	0.23–2.00	0.16–1.77	
A1A2										
Cases/controls	37/33	26/29	23/25	21/23						
OR	1.00	0.81	0.75	0.73	0.431	1.00	1.13	0.76	0.53	0.1277
95% CI	(Ref.)	0.38–1.73	0.34–1.63	0.33–1.59		(Ref.)	0.48–2.68	0.31–1.90	0.21–1.32	
A2A2										
Cases/controls	20/6	12/8	7/14	5/13						
OR	1.00	0.68	0.14	0.06	0.001	1.00	0.15	0.02	0.01	0.0015
95% CI	(Ref.)	0.16–2.84	0.03–0.70	0.01–0.35		(Ref.)	0.01–1.68	0.00–0.41	0.00–0.21	
Plasma genistein										
median, *nmol/L*	1.45	2.76	6.04	19.66						
CYP17 A1A1										
Cases/controls	16/19	18/19	15/22	19/24						
OR	1.00	1.24	0.65	0.97	0.877	1.00	1.47	0.64	0.84	0.5870
95% CI	(Ref.)	0.46–3.37	0.24–1.75	0.37–2.53		(Ref.)	0.45–4.75	0.20–2.06	0.28–2.50	
A1A2										
Cases/controls	23/30	19/31	38/21	27/26						
OR	1.00	0.77	2.26	1.25	0.57	1.00	0.92	2.41	1.23	0.7177
95% CI	(Ref.)	0.34–1.76	0.99–5.12	0.56–2.76		(Ref.)	0.35–2.39	0.94–6.14	0.49–3.10	
A2A2										
Cases/controls	10/9	6/9	12/14	16/9						
OR	1.00	0.16	0.28	1.38	0.136	1.00	0.02	0.02	3.17	0.0370
95% CI	(Ref.)	0.03–1.08	0.06–1.28	0.33–5.77		(Ref.)	0.00–0.49	0.00–0.54	0.36–28.10	

[1] Adjusted for age at menarche, use of oral contraceptives, family history of breast cancer, number of births, duration of breastfeeding, education, alcohol consumption, and BMI. OR, odds ratio; Ref., reference group.

enterolignans, and the plasma concentration of ENL may not be unexpected but nevertheless reassuring. These findings corroborate the results reported by McCann et al. (34) of a significant protective effect of high levels of calculated intestinal ENL and enterodiol production from ingested food on breast cancer risk for A2 carriers but not A1A1 homozygotes in premenopausal women only.

Some possible limitations should nevertheless be discussed when interpreting the results. Both studies were medium sized so that stratification by CYP17 genotype resulted in relatively small subgroup numbers. Although this usually increases the probability of chance findings, the consistency of the results from the 2 studies lends greater credibility to a possible modifying effect of CYP17 genotype. Secondly, case-control studies are subject to recall bias, especially when regarding dietary intake. Furthermore, the activity of the individual gut microflora, which affects the bioavailability of lignans, is unknown, and the calculation of dietary lignan intake, as well as of intestinally formed enterolignans, suffers from major limitations (42,43). The use of plasma concentrations as biomarkers of intake and subsequent intestinal metabolism may be regarded as a more valid method to assess the association between phytoestrogens and breast cancer risk. However, plasma concentrations measure intake at one point in time, whereas dietary intake calculations are based on regular intake over a whole year. Plasma concentrations are also more sensitive to dietary changes subsequent to cancer diagnosis and treatment. The strength of this study lies in the use of the different measures for estimating phytoestrogen intake.

We did not observe CYP17 effect modification for genistein, although a significant inverse association between dietary genistein intake and premenopausal breast cancer risk was observed in the original study population, which was not confirmed using plasma genistein concentrations (4,35). However, the effect of genistein on breast cancer risk at such low concentrations appears questionable (44), such that misclassification by exposure group seems a more likely explanation.

The biological mechanism by which phytoestrogens, particularly lignans in the Western diet, can protect against hormone-dependent breast cancer is likely through their competitive effects on the generation, transport, and removal of endogenous steroid hormones. Most studies have investigated the effect of isoflavone consumption and demonstrated its potential to modify steroid hormone levels and the length of the menstrual cycle (45–47). Lignan supplementation has also been shown to significantly increase the urinary 2-hydroxyestrogen:16α-hydroxyestrone (2:16α-OHE_1) ratio in both premenopausal and postmenopausal women, which is hypothesized to be associated with a decreased risk of breast cancer (48,49).

The observation that the inverse association of breast cancer risk with lignans differs according to CYP17 genotype appears plausible, although the effect of the CYP17 genotype itself on the risk of breast cancer has been considered to be, at most, weak (26). The A2 allele of the CYP17 has been associated with higher levels of dehydroepiandrosterone (DHEA), as well as hormones derived from it, such as dehydroepiandrosterone

TABLE 5

Crude and adjusted OR and 95% CI for the risk of premenopausal breast cancer by phytoestrogen intake and CYP17 genotype (n = 840)

	Quartiles, crude results					Quartiles, adjusted results[1]				
	1	2	3	4	*P* for trend	1	2	3	4	*P* for trend
Enterolactone (calculated)										
CYP17 A1A1										
Cases/controls	17/60	18/49	22/45	21/55						
OR	1.00	2.09	1.57	1.39	0.747	1.00	2.47	1.28	0.96	0.3437
95% CI	(Ref.)	1.01–4.33	0.74–3.34	0.65–2.96		(Ref.)	1.03–5.96	0.51–3.26	0.34–2.71	
A1A2										
Cases/controls	33/63	30/69	33/67	30/63						
OR	1.00	0.81	0.86	0.86	0.745	1.00	0.76	0.68	0.65	0.2924
95% CI	(Ref.)	0.44–1.50	0.47–1.59	0.47–1.59		(Ref.)	0.39–1.54	0.34–1.37	0.31–1.39	
A2A2										
Cases/controls	12/20	20/25	11/32	10/25						
OR	1.00	1.22	0.40	0.54	0.089	1.00	0.88	0.26	0.21	0.0119
95% CI	(Ref.)	0.45–3.30	0.14–1.16	0.19–1.53		(Ref.)	0.26–3.02	0.07–0.95	0.05–0.88	
Enterodiol (calculated)										
CYP17 A1A1										
Cases/controls	20/54	25/50	23/50	20/55						
OR	1.00	1.48	1.28	0.97	0.631	1.00	1.27	1.03	0.60	0.1442
95% CI	(Ref.)	0.71–3.06	0.62–2.63	0.45–2.05		(Ref.)	0.54–3.02	0.43–2.47	0.23–1.56	
A1A2										
Cases/controls	30/66	33/73	31/58	32/65						
OR	1.00	0.96	1.08	1.08	0.719	1.00	0.82	1.06	1.00	0.7846
95% CI	(Ref.)	0.52–1.77	0.58–2.02	0.58–2.02		(Ref.)	0.42–1.61	0.53–2.14	0.48–2.07	
A2A2										
Cases/controls	18/23	11/20	13/36	11/23						
OR	1.00	0.55	0.31	0.47	0.185	1.00	0.47	0.31	0.31	0.0794
95% CI	(Ref.)	0.20–1.51	0.11–0.85	0.17–1.29		(Ref.)	0.15–1.48	0.10–0.98	0.09–1.09	
Matairesinol intake										
CYP17 A1A1										
Cases/controls	26/58	16/52	28/50	18/49						
OR	1.00	0.65	1.27	0.80	0.879	1.00	0.55	0.72	0.47	0.1960
95% CI	(Ref.)	0.31–1.35	0.65–2.48	0.38–1.68		(Ref.)	0.23–1.29	0.31–1.67	0.18–1.26	
A1A2										
Cases/controls	38/68	29/69	24/63	35/62						
OR	1.00	0.82	0.67	1.03	0.874	1.00	0.71	0.58	0.85	0.7052
95% CI	(Ref.)	0.45–1.50	0.36–1.26	0.57–1.85		(Ref.)	0.37–1.38	0.29–1.15	0.30–1.80	
A2A2										
Cases/controls	14/17	17/22	9/31	13/32						
OR	1.00	0.68	0.21	0.37	0.061	1.00	0.60	0.18	0.27	0.0384
95% CI	(Ref.)	0.25–1.88	0.07–0.69	0.13–1.03		(Ref.)	0.19–1.93	0.04–0.69	0.07–1.00	
Genistein intake										
CYP17 A1A1										
Cases/controls	33/52	19/53	20/54	16/50						
OR	1.00	0.55	0.58	0.53	0.160	1.00	0.52	0.44	0.46	0.1783
95% CI	(Ref.)	0.28–1.11	0.29–1.15	0.25–1.12		(Ref.)	0.22–1.23	0.19–1.04	0.18–1.16	
A1A2										
Cases/controls	37/63	30/59	32/68	27/72						
OR	1.00	0.78	0.77	0.57	0.084	1.00	0.75	0.59	0.41	0.0236
95% CI	(Ref.)	0.42–1.45	0.43–1.39	0.31–1.05		(Ref.)	0.39–1.46	0.30–1.17	0.19–0.87	
A2A2										
Cases/controls	12/28	18/31	10/22	13/21						
OR	1.00	1.72	1.37	1.43	0.749	1.00	1.62	1.01	0.85	0.5335
95% CI	(Ref.)	0.64–4.59	0.43–4.34	0.51–4.05		(Ref.)	0.48–5.47	0.22–4.62	0.18–4.14	

[1] Adjusted for age at menarche, use of oral contraceptives, family history of breast cancer, number of births, duration of breastfeeding, education, alcohol consumption, BMI, and deciles of total daily energy intake. OR, odds ratio; Ref., reference group.

sulfate (DHEAS), androstenedione, estradiol, and estrones in premenopausal as well as postmenopausal women, in some (21,22) but not all studies (50,51). The protective effect of lignans may have a detectable impact only in women who have higher concentrations of endogenous hormones and presumably a higher breast cancer risk. These results do not have to be explained by a direct interaction between lignans and CYP17. Due to the structural similarity between estrogens and phytoestrogens, phytoestrogens may act as substrates and alter the activity of enzymes involved in the biosyntheses or metabolisms of estrogens downstream of CYP17, including aromatase (31), 17β-hydroxysteroid dehydrogenase (32), and steroid sulfotransferases (33), and thereby reducing the concentration of active estrogens. In view of these results, further studies regarding the mechanism of action between lignans and steroid metabolism are warranted.

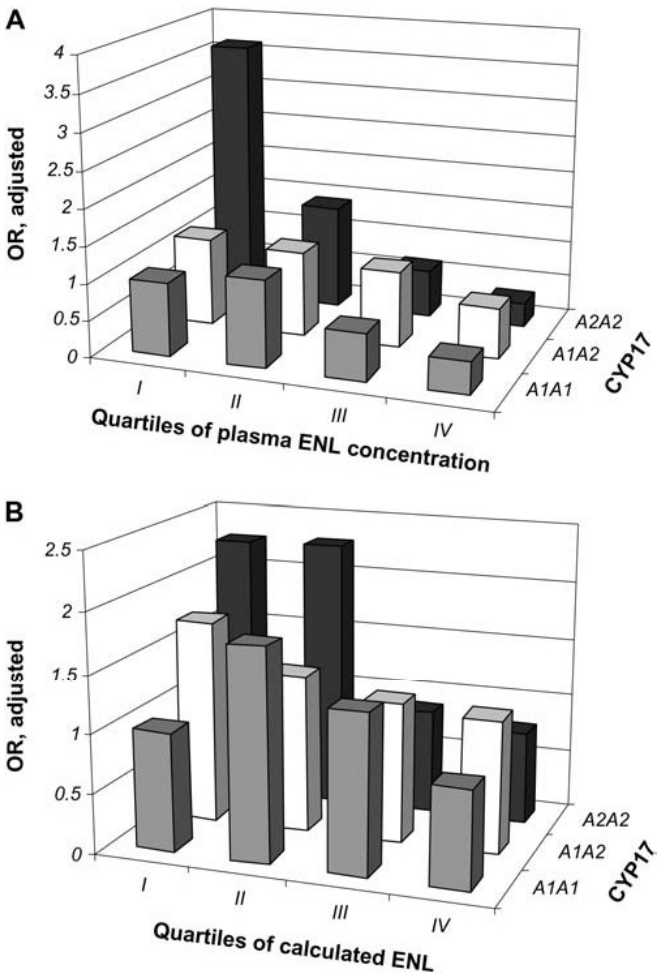

FIGURE 1 Adjusted odds ratios (OR) for the risk of premenopausal breast cancer by plasma enterolactone (ENL) concentrations and CYP17 genotype ($n = 454$) (*A*) and calculated amount of intestinally produced ENL and CYP17 genotype ($n = 840$) (*B*). (For adjustment variables see Tables 4, 5.)

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