Oral Contraceptive Use and Breast Cancer Risk: Modification by NAD(P)H:Quinone Oxoreductase (*NQO1*) Genetic Polymorphisms

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Abstract

Despite intensive study, the relationship between oral contraception (OC) and breast cancer remains unclear. OCs contain a potent synthetic estrogen (ethinyl estradiol) but lower endogenous estradiol levels, and ethinyl estradiol is a weak progenitor of semiquinones, catechol estrogens capable of damaging DNA. NAD(P)H:quinone oxoreductase (*NQO1*) stabilizes semiquinones, thus potentially preventing genetic damage from catechol estrogens, and the *NQO1 C609T* polymorphism seems functionally relevant. Using data from the Shanghai Breast Cancer Study, a population-based case-control study, we investigated the relationships between OC use (20% ever using), breast cancer, and *NQO1* (*C/C* 31% and *C/T* + *T/T* 69%) among 1,039 cases and 1,121 controls. Breast cancer was not significantly associated with *NQO1* genotype. There was a significant protective association between OC after age 30 years and premenopausal breast cancer [odds ratio (OR) 0.51, 95% confidence interval (95% CI) 0.29-0.89] primarily with the *NQO1 T* allele (*C/C* OR 0.76, 95% CI 0.31-1.82; *C/T* + *T/T* OR 0.38, 95% CI 0.18-0.80; *P* for interaction = 0.19). The association between premenopausal breast cancer and OCs significantly differed with *NQO1* genotype when using OCs for >18 months (*C/C* OR 2.34, 95% CI 0.92-5.99; *C/T* + *T/T* OR 0.69, 95% CI 0.38-1.25; *P* for interaction = 0.02). Among women with the *C/C* genotype, postmenopausal breast cancer was significantly associated with ever-using OCs (*C/C* OR 2.01, 95% CI 1.08-3.74; *C/T* + *T/T* OR 0.72, 95% CI 0.49-1.05; *P* for interaction < 0.01). This crossover was stronger with OC use prior to age 30 years (*C/C* OR 3.00, 95% CI 1.43-6.25; *C/T* or *T/T* OR 0.49, 95% CI 0.29-0.81; *P* for interaction < 0.01). Our results require confirmation but suggest that the OC and breast cancer association depends on the ability to invoke protection from catechol estrogens. (Cancer Epidemiol Biomarkers Prev 2004;13(8):1308–15)

Introduction

Estrogens stimulate breast cell proliferation, and established breast cancer risk factors such as early age at menarche, later menopause, and hormone replacement therapy are indicative of greater lifetime estrogen exposure. Most oral contraceptives (OC) contain ethinyl estradiol, a potent synthetic estrogen with higher affinity for the estrogen receptor and longer persistence in the body than estradiol (1-3). However, prior studies of the OC and breast cancer association have not been consistent, despite this high estrogenic potential. Several studies, including a pooled analysis combining data from 54 studies, reported no association between past OC and breast cancer (4, 5), whereas OC may lead to earlier diagnosis consistent with mammography screening among OC users (5, 6) or lower breast cancer mortality (7). Alternatively, breast cancer risk may increase with OC at younger ages (8) among women with *BRCA1* mutations (9) or family breast cancer history (10) or affect only lobular breast cancer (11). Whereas reproductive factors are consistently associated with breast cancer, the partial disconnection between exogenous ethinyl estradiol exposure during the reproductive years and breast cancer suggests that synthetic estrogens and endogenous estrogens affect breast carcinogenesis through different mechanisms.

The metabolism of estradiol and ethinyl estradiol substantively differ such that OCs could shift the constellation of biologically active estrogens in the body. In general, estradiol is converted to estrone and irreversibly hydroxylated at C-16, C-4, or C-2 (Fig. 1; refs. 12, 13). The 16-hydroxyestrone metabolite has a greater affinity for the estrogen receptor compared with the 4-hydroxyestrone or 2-hydroxyestrone metabolites (14, 15). 2-Methoxyestrone may inhibit breast carcinogenesis (15, 16), and several studies, although not all, have reported that higher urinary 2-hydroxyestrone

Received 11/18/03; revised 3/18/04; accepted 3/29/04.

Grant support: National Cancer Institute grants RO1 CA64277 and RO1 CA90899.

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Note: J.H. Fowke, the primary author, developed the hypothesis and conducted the statistical analysis. W. Zheng, the principal investigator, obtained the research funding for the SBCS. W. Zheng and X-O. Shu are responsible for the SBCS study design. Q. Dai coordinated SBCS field operations, and Qiuyin Cai developed the *NQO1* genotyping assay, reviewed the analytic approaches, and contributed to manuscript writing. Y-T. Gao, the principal investigator for the subcontract of the SBCS in Shanghai Cancer Institute, played a critical role, along with F. Jin, in all data collection and recruitment protocols.

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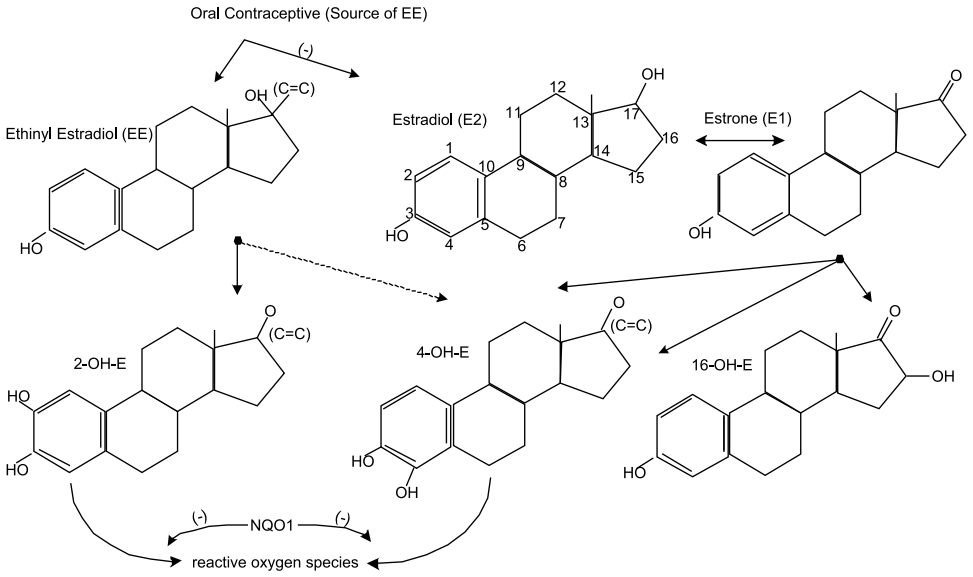

Figure 1. Estradiol and ethinyl estradiol metabolism.

levels relative to 16-hydroxyestrone were associated with breast cancer risk (17-23). However, the 4-hydroxyestrone and 2-hydroxyestrone catechol (*ortho*-hydroquinone) estrogens redox cycle through a reactive semiquinone intermediary and bind to DNA (24). OCs suppress endogenous estradiol release (3, 25), and the ethinyl group on C-17 of ethinyl estradiol blocks C-17 and C-16 oxidation (26). Thus, the 2-hydroxy-ethinyl estradiol and 4-hydroxy-ethinyl estradiol metabolites are the major and minor metabolic products, respectively, and ethinyl estradiol metabolites are more persistent than estradiol metabolites (26-28). Overall, OC decreases endogenous estradiol release and shifts the composition of estrogen metabolites previously associated with breast cancer risk.

The NAD(P)H:quinone oxoreductase (*NQO1*) may have a critical role in protecting DNA from reactive catechol estrogens through a two-electron reduction of estrogen semiquinones (29). *NQO1* expression is partially regulated by the estrogen receptor (30), and *NQO1* suppression increases estradiol-dependent tumor formation in animal models (31, 32). The *NQO1 C609T* genetic polymorphism leads to three *NQO1* functional phenotypes *in vitro* (33) and decreases NQO1 protein expression in human tissue (34).

There have been few opportunities to investigate genetic susceptibility to OC (9), and analysis of the Chinese population presents a unique opportunity. Compared with most Western countries, OCs have been available at little or no expense to the individual, and fewer OC formulas have been available. Two prior Asian investigations of OC and breast cancer found OC after age 45 years (n = 534 cases; ref. 35) or before age 25 years (n = 174 cases; ref. 36) associated with breast cancer. Using data from the Shanghai Breast Cancer Study (SBCS), a large population-based case-control study, we investigated the OC and breast cancer relationship across *NQO1 C609T* genotypes, possibly determining susceptibility to reactive estrogen metabolites.

Methods

Participants. Details of the SBCS have been reported (37), and all relevant institutional review boards have approved all protocols. Breast cancer cases diagnosed between August 1996 and March 1998 were identified through a rapid case ascertainment system throughout the major hospitals in Shanghai and supplemented by the population-based Shanghai Tumor Registry. Of the 1,602 eligible breast cancer cases, data collection interviews were completed for 1,459 (91.1%; refusals n = 109, 6.8%; death prior to interview n = 17, 1.1%; inability to locate n = 17, 1.1%). Two senior pathologists confirmed all cancer diagnoses, and >95% of tumors were of ductal origin.

Controls were women randomly selected from the Shanghai Resident Registry, a registry of all adult residents in urban Shanghai. The age distribution of selected controls was frequency matched (5-year intervals) to the expected age distribution of the breast cancer cases. Eligible women had to live at the registered address between 1996 and 1998 and were between ages 25 and 70 years. In-person interviews were completed for 1,556 (90.2%) of the 1,724 eligible population controls (refusals n = 166, 9.6%; death or prior cancer diagnosis n = 2, 0.1%).

All participants were interviewed in-person by trained interviewers and measured for weight, waist and hip circumferences, and height. Demographics, reproductive history, use of hormone replacement therapy, and other data were measured by a structured questionnaire. Subjects were asked if they had ever taken OCs, injected contraceptives, or other forms of birth control. Subjects ever taking any OC were queried for age at first use, duration of use, age at last use, and the name of the OC used for each episode. Of those completing the in-person interviews, 2,503 (82% of cases and 84% of controls) donated a blood sample.

Genotyping Methods. Genomic DNA was extracted from blood buffy coat fractions (Puregene DNA Isolation

Kit, Gentra Systems, Minneapolis, MN). The *NQO1 C609T* polymorphism, leading to a $Pro^{187}Ser$ exchange, was evaluated by the PCR-RFLP method. The primers for the PCR reaction were F: 5′-TCCTCAGAGTGG-CATTCTGC-3′ and R: 5′-TCTCCTCATCCTG-TACCTCT-3′. Each PCR product was subjected to *Hin*fI digestion. The C-to-T substitution at nucleotide 609 creates a *Hin*fI restriction site. The PCR product (230 bp) with *C* allele was digested to two fragments (195 and 35 bp), whereas the PCR product with *T* allele was digested to three fragments (151, 44, and 35 bp). Laboratory staff was blind to the subject identify, and quality control samples were included in genotyping all assays. Each 96-well plate contained one water, two Centre d'Etude du Polymorphisme Humain (1347-02) DNA, two blinded quality control DNA, and two unblinded quality control DNA samples. Genotyping was successful for 2,361 (94.3%) of 2,503 subjects providing a blood sample. The *NQO1 C609T* polymorphism was within Hardy-Weinberg equilibrium ($P = 0.63$) with *C/C*, *C/T*, and *T/T* genotype frequencies of 30.7% ($n = 664$), 49.9% ($n = 1,077$), and 19.3% ($n = 419$), respectively.

Statistical Analysis. Prior analysis of the SBCS found that breast cancer risk significantly associated with an earlier age at menarche, later age at menopause, later age at first live birth, and a nonphysically active lifestyle (38). Women reporting ever using hormone replacement or estrogen therapy ($n = 143$), injected contraception ($n = 53$), or both ($n = 7$) were excluded. The final data set contained 2,160 subjects, including 1,418 premenopausal and 742 postmenopausal women with *NQO1* genotype data. Age, body mass index (BMI), income, and reproductive factors did not substantively differ between these participants and the remaining SBCS participants.

Several indices of OC were created, including ever/never (at least 1 month of use), duration of use, number of episodes (indicating sporadic use over several time intervals), current use at time of study enrollment, age at first use, age at last use, time since first use, and time since last use. The Wilcoxon rank sum test was used to compare OC use patterns between cases and controls. Spearman correlation coefficients characterized the associations between continuous indices of OC use. Odds ratios (OR) and 95% confidence intervals (95% CI) summarizing the association between OC and breast cancer were calculated using multivariable logistic regression (SAS Institute, Inc., Cary, NC), adjusting for age, BMI, parity, fibroadenoma history, age at menarche, leisure time physical activity, education, and age at menopause (postmenopausal only). Logistic regression analyses were repeated among participants within each *NQO1* genotype (*C/C*, *C/T*, *TT*, or *C/T* + *TT*). OC and breast cancer associations within the *T/T* genotype were comparable with analyses within the *C/T* genotype, and the *C/T* and *T/T* genotypes were combined to improve stability. *P* values for interaction between *NQO1* genotype and OC indices were determined by inserting a cross-product term, with main-effect terms, into the logistic model.

Results

The majority of participants in this population-based breast cancer case-control study were premenopausal at study entry (Table 1). The average BMI was 22.8 and 24.4 kg/m^2 for premenopausal and postmenopausal women, respectively, and few participants reported ever using tobacco or a family history of breast cancer. Most subjects had less than a college education but participated in leisure time physical activity. Premenopausal women reported having fewer children than postmenopausal women.

Postmenopausal women were more likely to report ever taking OCs compared with premenopausal women (postmenopausal 31.9%, premenopausal 14.5%; $P < 0.01$; Table 1). Only eight premenopausal subjects were taking OCs at the time of study entry (two cases and six controls), and only one subject reported taking OCs before age 20 years. Table 2 further summarizes OC use by menopausal and case-control status. Premenopausal cases and controls started taking OCs at approximately the same age and for a similar duration. More time had passed since first or last taking OCs among premenopausal cases; however, premenopausal cases were ~1.5 years older than premenopausal controls. For most women, OCs were taken during a single episode, and patterns of OC did not substantially differ between postmenopausal cases and controls.

Overall, OC use alone was not associated with premenopausal breast cancer (Table 3). However, taking OCs after age 30 years was significantly associated with lower premenopausal breast cancer risk (OR 0.51, 95% CI 0.29-0.89). Postmenopausal breast cancer risk was not significantly associated with OC. There was no evidence of an association between ever-using OCs and breast cancer among subjects with a family breast cancer history (OR 1.15, 95% CI 0.34-3.81; OC 9 cases and 6 controls, non-OC 26 cases and 21 controls; premenopausal and postmenopausal subjects combined; adjusted

Table 1. Study population description

	Premenopausal women ($n = 1,418$)	Postmenopausal women ($n = 742$)
Age (y), mean (SD)	42.6 (5.1)	56.4 (5.6)
BMI, mean (SD)	22.8 (3.2)	24.4 (3.7)
Fibroadenoma, *n* (%)	111 (7.87)	46 (6.2)
Family breast cancer, *n* (%)	36 (2.5)	26 (3.5)
Age at menarche (<11 y), *n* (%)	17 (1.2)	12 (1.6)
No physical activity, *n* (%)	210 (14.8)	269 (36.3)
Age at menopause (>55 y), *n* (%)		65 (8.8)
Ever smoke, *n* (%)	24 (1.7)	32 (4.3)
Parity, *n* (%)		
≥2 children	159 (11.2)	541 (72.9)
1 child	1,191 (84.0)	174 (23.5)
No children	68 (4.8)	27 (3.7)
Income (yuan), *n* (%)		
<10,000	133 (9.4)	105 (14.2)
10,000-20,000	572 (40.3)	300 (40.4)
20,000-30,000	403 (28.4)	167 (22.5)
>30,000	310 (21.9)	170 (22.9)
Education, *n* (%)		
Less than high school	779 (55.0)	458 (61.7)
High school	508 (35.6)	185 (24.9)
College/professional	131 (9.2)	99 (13.4)
Ever use OC	206 (14.5)	237 (31.9)
Current OC use	8 (0.6)	0 (0)

Table 2. Median analysis of OC use among women ever taking OC, the SBCS

	Premenopausal women					Postmenopausal women				
	Cases (n = 103)		Controls (n = 103)		P*	Cases (n = 110)		Controls (n = 127)		P
	Median	Q1, Q3	Median	Q1, Q3		Median	Q1, Q3	Median	Q1, Q3	
Duration of use (mo)	10	3, 36	12	4, 48	0.61	24	10, 60	24	12, 84	0.65
No. of OC episodes†	1	1, 1	1	1, 1	0.35	1	1, 1	1	1, 1	0.61
Age at first use (y)	28	26, 30	29	27, 32	0.13	30	27, 33	30	27, 32	0.65
Time since first use (y)	18	15, 21	15	12, 19	<0.01	26	23, 28	26	23, 30	0.31
Age at last use (y)	30	27, 33	32	28, 35	0.05	33	30, 38	33	30, 40	0.76
Time since last use (y)	16	11, 20	14	8, 16	<0.01	22	17, 26	22	17, 27	0.81

*P from Wilcoxon rank sum test.
†An OC episode reflects a period of time subjects use OCs. Women reporting ever using OCs have at least one episode, and a new episode occurs if a women stops using OCs for at least 1 month then starts to take OCs again at a later time.

for age and other factors). In addition, calendar year first taking OCs was not significantly associated with premenopausal or postmenopausal breast cancer (data not shown).

The *NQO1 C607T* polymorphism was not significantly associated with premenopausal [*CC* (reference); *CT* OR 0.87, 95% CI 0.68-1.11; *TT* OR 1.06, 95% CI 0.78-1.44] or postmenopausal [*CC* (reference); *CT* OR 0.92, 95% CI 0.65-1.29; *TT* OR 0.92, 95% CI 0.60-1.40] breast cancer.

Table 4 summarizes OC and premenopausal breast cancer by *NQO1* genotype. There was little association between premenopausal breast cancer and ever-using OC among women with the *T* allele of *NQO1* (OR 0.85, 95% CI 0.58-1.25). With a longer duration of OC, there was a marginal association with premenopausal breast cancer among subjects with the *C/C* genotype (OR 2.34, 95% CI 0.92-5.99). We note that the small number of women reporting any OC use limits statistical inferences; however, the interaction across *NQO1* genotypes was significant (P = 0.02). In addition, OC after age 30 years was significantly protective for premenopausal breast cancer among women with the *NQO1 T* allele. OC indices representing age at first use and time since first taking OC were correlated ($r_s = -0.46$, $P < 0.01$), and the protective associations for age-related and time-related indices were similar (data not shown). Unlike the analysis of duration of use, interactions with *NQO1* genotype and age of OC use were not statistically significant.

Table 3. Indices of OC and breast cancer, the SBCS

OC exposure	Premenopausal women			Postmenopausal women		
	Cases, n (%)	Controls, n (%)	OR* (95% CI)	Cases, n (%)	Controls, n (%)	OR (95% CI)
Never use OCs (reference)	587 (85)	625 (86)	1.00	239 (68)	266 (68)	1.00
Ever use OCs	103 (15)	103 (14)	0.92 (0.67-1.26)	110 (32)	127 (32)	0.96 (0.70-1.32)
Duration of OC use (y)						
<2	63 (9)	67 (9)	0.88 (0.60-1.28)	47 (13)	54 (14)	0.90 (0.58-1.41)
≥2	40 (6)	36 (5)	1.00 (0.62-1.62)	63 (18)	73 (19)	0.99 (0.67-1.46)
No. of OC episodes†						
1	99 (14)	96 (13)	0.94 (0.68-1.30)	104 (30)	118 (30)	0.96 (0.69-1.34)
2-3	4 (1)	7 (1)	0.55 (0.15-1.92)	6 (2)	9 (2)	0.81 (0.27-2.36)
Age at first OC use (y)						
≤30	80 (12)	67 (9)	1.17 (0.81-1.68)	60	77 (19)	0.89 (0.59-1.32)
>30	23 (3)	36 (5)	0.51 (0.29-0.89)	50 (14)	50 (13)	1.05 (0.67-1.63)
Time since first OC use (y)‡						
0-15 (20)	32 (5)	17 (2)	0.80 (0.57-1.13)	18 (5)	16 (4)	1.07 (0.52-2.22)
>15 (20)	71 (10)	86 (12)	1.58 (0.83-3.02)	92 (26)	111 (28)	0.93 (0.67-1.31)
Age at last OC use (y)						
≤30	53 (8)	41 (6)	1.20 (0.77-1.87)	34 (10)	35 (9)	1.11 (0.65-1.90)
>30	50 (7)	62 (9)	0.71 (0.47-1.07)	76 (22)	92 (23)	0.88 (0.62-1.27)
Time since last OC use (y)						
0-15	44 (6)	66 (9)	1.36 (0.86-2.15)	23 (7)	28 (7)	0.98 (0.69-1.39)
>15	59 (9)	37 (5)	0.68 (0.46-1.03)	87 (25)	99 (25)	0.87 (0.48-1.57)
OC use before first pregnancy						
Before	10 (2)	13 (2)	0.85 (0.36-2.02)	1 (0)	2 (1)	0.43 (0.04-4.85)
After	193 (14)	90 (13)	0.94 (0.68-1.31)	109 (32)	125 (33)	0.96 (0.69-1.32)

*OR adjusted for age, BMI, parity, education, fibroadenoma history, age at menarche, leisure time activity, age at first live birth, and age at menopause (postmenopausal only).
†An OC episode reflects a period of time subjects use OCs. Women reporting ever using OCs have at least one episode, and a new episode occurs if a women stops using OCs for at least 1 month then starts to take OCs again at a later time.
‡Time since first taking OCs (20): for postmenopausal women, the analysis used a time of 20 years since first taking OC.

Table 4. Premenopausal breast cancer and OC by *NQO1* genotype, the SBCS

NQO1	Cases/Controls	OR (95% CI)	Cases/Controls	OR* (95% CI)
	Never used OCs (reference)†		Ever used OCs	
CC	178/190	1.0	39/29	1.06 (0.60-1.88)
CT/TT	409/435	1.0	103/74	0.85 (0.58-1.25)
P for interaction				0.30
Duration of OC use (mo)	≤18		>18	
CC	20/22	0.68 (0.33-1.35)	19/7	2.34 (0.92-5.99)
CT/TT	43/45	0.96 (0.61-1.51)	21/29	0.69 (0.38-1.25)
P for interaction		0.48		0.02
Age at first OC use (y)	≤30		>30	
CC	27/17	1.30 (0.64-2.60)	12/12	0.76 (0.31-1.82)
CT/TT	53/50	1.12 (0.73-1.73)	11/24	0.38 (0.18-0.80)
P for interaction		0.53		0.19
Age at last OC use (y)	≤30		>30	
CC	14/10	1.08 (0.42-2.76)	25/19	1.05 (0.54-2.05)
CT/TT	39/31	1.34 (0.80-2.23)	25/43	0.53 (0.31-0.91)
P for interaction		0.82		0.06

*All ORs adjusted for age, BMI, education, parity, fibroadenoma history, age at menarche, and leisure activity.
†Reported never taking OCs within *NQO1* genotype serves as the reference group for all associations.

Among postmenopausal women (Table 5), ever-taking OC was significantly associated with a 2-fold greater breast cancer risk among women with the *C/C* genotype (OR 2.01, 95% CI 1.08-3.74). In contrast, OC was marginally protective among women with the *T* allele (OR 0.72, 95% CI 0.49-1.05), and the difference between these genotypes was significant (*P* for interaction < 0.01). This crossover pattern was stronger and significant when restricted to women taking OC for <18 months or prior to age 30 years. There was no clear association among postmenopausal breast cancer, *NQO1*, and OC after age 30 years. Duration using OCs was strongly correlated with age at last use ($r_s = 0.62$, $P < 0.01$) but not with age at first use ($r_s = -0.05$, $P = 0.38$), suggesting that duration taking OC was dependent on the age OC use ended.

Discussion

Consistent with most studies (5, 6, 35, 36), we found little evidence that OC alone increased breast cancer risk among women living in Shanghai, China. Instead, the association between OC and breast cancer was specific to menopausal status at diagnosis, *NQO1 C609T* genotype, age at use or time since use, and perhaps duration of use. Across both premenopausal and postmenopausal women, OC was associated with a lower breast cancer risk among women with the *T* allele of *NQO1*, and these associations were stronger with taking OCs at older (premenopausal) or younger (postmenopausal) ages. OC before age 30 years was associated with increased postmenopausal breast cancer among women with the *NQO1 C/C* genotype, whereas taking OCs >18 months marginally increased premenopausal breast cancer risk among women with the *NQO1 T* allele. These results provide supportive evidence that the mechanisms responsible for any association between OC and breast cancer may include products of estradiol and estrone metabolism.

As illustrated in Fig. 1, initial metabolism of estradiol primarily follows 17-hydroxysteroid dehydrogenase–mediated conversion to estrone; *P*450-dependent hydroxylation at C-2, C-4, or C-16; and conjugation (e.g., catechol-*O*-methyl transferase; refs. 12, 13). The catechol

Table 5. Postmenopausal breast cancer and OC by *NQO1* genotype, the SBCS

NQO1	Cases/Controls	OR (95% CI)	Cases/Controls	OR* (95% CI)
	Never used OCs (reference)†		Ever use	
CC	72/93	1.0	38/25	2.01 (1.08-3.74)
CT/TT	167/173	1.0	72/102	0.72 (0.49-1.05)
P for interaction				<0.01
Duration of OC use (mo)	≤18		>18	
CC	15/6	3.37 (1.19-9.51)	23/19	1.60 (0.79-3.25)
CT/TT	32/48	0.63 (0.38-1.06)	40/54	0.79 (0.49-1.27)
P for interaction		<0.01		0.21
Age at first OC use (y)	≤30		>30	
CC	31/14	3.00 (1.43-6.25)	7/11	0.82 (0.29-2.32)
CT/TT	29/63	0.49 (0.29-0.81)	43/39	1.06 (0.64-1.74)
P for interaction		<0.01		0.19
Age at last OC use (y)	≤30		>30	
CC	17/4	6.08 (1.86-19.71)	21/21	1.29 (0.64-2.62)
CT/TT	17/31	0.59 (0.30-1.13)	55/71	0.78 (0.50-1.18)
P for interaction		<0.01		0.53

*All ORs adjusted for age, BMI, education, parity, fibroadenoma history, age at menarche, leisure activity, and age at menopause.
†Reported never taking OCs within *NQO1* genotype serves as the reference group for all associations.

estrogens (i.e., 2-hydroxyestrone and 4-hydroxyestrone) have less affinity for the estrogen receptor than 16-hydroxyestrone (39), and the methylated conjugate of 2-hydroxyestrone may inhibit angiogenesis (16). However, the catechol estrogens are unstable and redox cycle through a highly reactive semiquinone intermediary with genotoxic potential (15, 24). Breast tumors have higher 4-hydroxyestrone metabolite levels compared with normal tissue (16), and the 4-hydroxyestrone and 16-hydroxyestrone metabolites have been of particular interest in breast carcinogenesis (15, 24). OCs partially suppress estradiol release from the ovary (25, 40), and any differences between estradiol and ethinyl estradiol metabolism would indicate that OCs change the body estrogen metabolite composition.

Ethinyl estradiol is a minor progenitor of 16-hydroxyestrone and 4-hydroxyestrone metabolites, at least in the liver and kidney, and the 2-hydroxyestrone metabolite is the major metabolic product (26, 27). However, ethinyl estradiol administration enhanced oxidative DNA damage in a rodent tumor model (15), and sex hormone binding globulin and catechol-*O*-methyl transferase have a lower affinity for ethinyl estradiol and ethinyl estradiol metabolites compared with estradiol and estradiol metabolites (26, 27, 41, 42). Thus, OC perhaps increases breast cancer risk through exposure to a potent synthetic estrogen with high estrogen receptor affinity and persistence or decreases breast cancer risk by reducing 16-hydroxyestrone and 4-hydroxyestrone metabolite levels (27, 43).

Through a two-electron transfer, *NQO1* may prevent the redox cycling of catechol estrogens leading to genetic damage (32). With the *NQO1 T* allele, OC use was associated with reduced breast cancer risk, perhaps because cells without an adequate *NQO1* response have the capacity to invoke alternative metabolic enzymes or DNA repair mechanisms in response to a challenge. 4-Hydroxyestrone levels may decrease with ethinyl estradiol exposure, and the protective effect of this shift in estrogen metabolism may be easier to observe among those subjects with a weaker *NQO1* response and thus more susceptible to reactive oxygen species. In contrast to the protective associations observed within the *C/T* and *T/T* genotypes, postmenopausal breast cancer risk increased with OC among women with the *NQO1 C/C* genotype. This seems unlikely due simply to estrogen receptor activation, as OC usually precedes postmenopausal breast cancer diagnoses by ≥20 years. Importantly, *NQO1* is suppressed by estrogen receptor agonists such as estradiol, whereas antiestrogens such as tamoxifen induce a *NQO1* response (30-32). Thus, ethinyl estradiol with a high affinity for the estrogen receptor could increase susceptibility to reactive oxygen species by suppressing *NQO1*. This effect would be most evident among subjects with the functional *C/C* genotype and who may rely on *NQO1* to respond to a catechol estrogen challenge.

Long-term ethinyl estradiol and progestin exposure does not seem to be exceptionally toxic (44), whereas short-term ethinyl estradiol and progestin administration significantly reduced methylnitrosourea-induced rodent mammary tumor incidence (45). However, prior research investigating breast cancer and age, duration, or time since OC have been inconsistent (5-8, 46). There may be many reasons, and this study suggests that variations in genetic susceptibility to exogenous hormone exposure may contribute to prior observed inconsistencies. In this study, the low prevalence of OC use and the strong correlation among time, age, and duration of OC indices prevented isolation of separate effects for OC indices within *NQO1* genotype. Age using OCs was most consistently associated with breast cancer across these analyses; however, time since contraceptive use and diagnosis as well as duration also may be relevant.

Women using OCs may seek mammography leading to breast cancer diagnosis, and prior research has inconsistently controlled for mammography use. However, this is an unlikely explanation because only two cases were taking OC at time of diagnosis. Furthermore, screening mammography is less common in Shanghai compared with the United States, and OC use alone was not associated with greater breast cancer risk or stage at diagnosis (data not shown). In addition, we could not control for traditional Chinese medication use, although the relationships among traditional medicines, OCs, and *NQO1* genotype are not clear.

Our investigation among Chinese women living in Shanghai provides several advantages. All breast tumors diagnosed in Shanghai are reported to the Shanghai Tumor Registry regardless of taking OCs or *NQO1* genotype. In addition, ~80% of our cases were interviewed within 4 months of diagnosis, decreasing the likelihood of selection bias for these factors. Compared with the West, relatively few OCs were available in Shanghai. Over 75% of our participants reported using a single OC formulation (OC No. 1: 34 μg ethinyl estradiol, 0.625 mg norethindrone). The composition of OCs may change over time (8), but we found no relationship between breast cancer and calendar year of OC use. In the West, OCs are used to prevent a first birth, to prevent or delay additional births, to regulate menstrual cycle disorders, to decrease the risk of ovarian cysts and inflammatory disease, and for other clinical conditions (47). Most women in this study started using OCs after the birth of the first child, reducing variability in the OC and breast cancer association due to comorbidities in the study group or diverse reproductive characteristics. The prevalence of the *C609T* genetic polymorphism reported here (69.5%) and reported previously in northern Chinese (66.0%) is higher than among Caucasians (26%; ref. 34). Studies in populations with less variability in *NQO1* genotype and a larger number of OC formulas and birth patterns may require a larger sample size to identify a significant interaction among OC, breast cancer, and *NQO1*.

We hypothesized *a priori* that *NQO1* genotypes modified the OC and breast cancer association due to the plausible action of *NQO1* to stabilize catechol estrogens. However, many metabolic enzymes determine the constellation of estrogens in the body, and further investigation combining variations in *CYP1B1*, *COMT*, or other genes, alone or in combination with *NQO1*, is needed. In addition, OCs may affect breast cancer risk through alternative mechanisms. Ethinyl estradiol and progestins interact with estrogen, progesterone, and androgen receptors (27, 40, 48); transforming growth factor 1 and insulin-like growth factor 2 receptor pathways (49); insulin and lipid metabolism (50); and breast

tissue differentiation (45). Although the estrogenic pathways are clearly relevant (8), research on alternative mechanisms and the relationships with age and time of OC use are needed.

In summary, we report that the association between OC use and breast cancer risk is dependent on *NQO1* genotype and further varies with age of OC use and menopausal status. Our results require further investigation and confirmation but suggest a genetic susceptibility to OCs consistent with prior observations relating estrogen metabolism with breast cancer.

Acknowledgments

We thank Dr. Wenquin Wen for statistical consultation, Zhi-Xian Ruana and Jia-Rong Cheng for their valuable contribution to the field operation, Dr. Fritz Parl for several insightful discussions of estrogen metabolism and breast cancer, Drs. Aron Rosenthal and Yang Gong for information on OC in China, Sarah Hutchison for graphics support, and the SBCS participants for their generosity.

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Cancer Epidemiol Biomarkers Prev 2004;13:1308-1315.