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Award Number: DAMD17-98-1-8052

TITLE: P53, Environmental Risk Factors and Breast Cancer: A Population-Based Study

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REPORT DATE: September 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1999	Annual (01 Sep 98 - 31 Aug 99)

4. TITLE AND SUBTITLE
P53, Environmental Risk Factors and Breast Cancer: A Population-Based Study

5. FUNDING NUMBERS
DAMD17-98-1-8052

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The presence of *p53* mutations in tumor tissue have been hypothesized to represent a "fingerprint" of environmental carcinogens. As a first step in testing this hypothesis in breast cancer in humans, we are evaluating whether risk for women with tumors that show *p53* protein overexpression (*p53*+) assessed by immunohistochemistry in relation to certain environmental exposures, such as hormone replacement therapy, alcohol use, cigarette smoking, DDT levels in blood, or polycyclic aromatic hydrocarbons (PAH-DNA adducts), is higher than risk among women with tumors that show no *p53* protein overexpression (*p53*-), as compared with population-based controls. For this molecular epidemiology project, archived tumor tissue is being retrieved for the case participants of the NIH-funded parent study, the Long Island Breast Cancer Study Project. The retrieved archived tumor tissue is being cut and prepared to establish a tumor bank that can be linked to already collected risk factor data and stored samples of blood and urine. Two prepared slides per subject are being utilized for the p53 immunohistochemical assays. The lab data from the molecular epidemiology component will be coupled with the risk factor data on the respondents of the parent study to perform statistical analyses to evaluate the hypothesis of the molecular epidemiology component.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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Date

(4) TABLE OF CONTENTS

p53, Environmental Risk Factors and Breast Cancer:
A Population-Based Study

(5) INTRODUCTION

The presence of *p53* mutations in tumor tissue have been hypothesized to represent a "fingerprint" of environmental carcinogens. As a first step in testing this hypothesis in breast cancer in humans, we are evaluating whether risk for women with tumors that show *p53* protein overexpression (*p53+*) assessed by immunohistochemistry in relation to certain environmental exposures, such as hormone replacement therapy, alcohol use, cigarette smoking, DDT levels in blood, or polycyclic aromatic hydrocarbons (PAH-DNA adducts) in blood, is higher than risk among women with tumors that show no *p53* protein overexpression (*p53-*), as compared with population-based controls.

This study draws upon an ongoing population-based, case-control study, with the specific aims of determining whether breast cancer risk is related to blood levels of organochlorine compounds (including DDT, DDE, PCBs, and chlordane) or polycyclic aromatic hydrocarbons (PAH-DNA adducts). For the parent study, interviews were completed with 1,508 case women and 1,556 control women. For the offspring molecular epidemiology component, archived paraffin-embedded tumor tissue blocks are being retrieved for the 1,442 case women with signed medical record release forms from the 33 participating hospitals. The retrieved archived blocks are being prepared and cut at Columbia University for immunostaining for *p53* protein overexpression, and for storage for future molecular epidemiology studies. Laboratory results from the proposed study will be combined with the interview data, and laboratory results from blood samples, which are collected and analyzed as part of the parent study. The purpose of these combined statistical analyses is to determine whether the risk of *p53*-positive breast cancer in relation to certain environmental exposures (including hormone replacement therapy, alcohol use, cigarette smoking, DDT/DDE, PAH-DNA adducts, and others) is higher than risk for *p53*-negative breast cancer, as compared with population-based controls. Results from this study will help to identify a subgroup of women that may have tumors that are associated with environmental exposures. Future research efforts could then focus on this subgroup to identify signature *p53* mutations for the carcinogens.

(6) BODY

The grant application described the workscope of the study as follows. This three-year project includes four components: (1) retrieval of 1,442 paraffin-embedded tissue blocks from a population-based sample of breast cancer cases; (2) preparation of slides from the archived tissue for the planned assays, and to bank for future studies; (3) evaluation for evidence of *p53* overexpression in the archival tumor tissue by immunohistochemistry; and (4) estimation of the odds ratios for *p53* positive breast cancer in relation to environmental factors, information which is already being collected by the investigators. Most of the components of the study are to be conducted simultaneously, as described below.

Task 1. Retrieval of Paraffin-embedded Blocks, Months 1 - 32.

A. Request paraffin-embedded blocks from the appropriate hospitals.

B. After cutting slides from each block, return the block to the appropriate hospital.

C. Track on personal computer the collection and return of each block.

Task 2. Slide Preparation, Months 2 - 32.

A. Cut and prepare slides from the retrieved archived tissue according to the study protocol.

B. Bank tissue for future studies.

Task 3. Laboratory Analyses, Months 2 - 33.

A. Prepare slides from paraffin-embedded blocks.

B. Determine adequacy of tissue sample.

C. Evaluate tumor tissue for evidence of *p53* by immunohistochemistry.

D. Interpret and record immunohistochemical results.

Task 4. Data Entry and Statistical Analyses, Months 2-36.

A. Enter laboratory results into a SAS file on personal computer.

B. Merge (1) laboratory data that will be collected in the proposed study, and (2) the case and control data on risk factors that is already being collected and computerized by the investigators.

C. Determine, using SAS on personal computer, the odds ratios for breast cancer by *p53* status in relation to (1) hormone replacement therapy, (2) alcohol, (3) cigarette smoking, (4) DDT levels, (5) PAH-DNA adducts, and (6) other risk factors for breast cancer.

As of the end of year 1, all tasks have been initiated as follows. In our undertaking of these research activities, no unusual problems have been encountered to date.

Task 1. Of the 1,508 case women who participated in the parent study (the case-control interview of the Long Island Breast Cancer Study Project), 1,442 signed a medical record release form, which gives us access to her archived pathology specimens. Initiation of the block retrieval was delayed due to delays in the field work of the parent study. For the parent study, medical records were collected from each of the 33 participating hospitals at the completion of the field work. In an effort to reduce the volunteer labor of the participating hospitals, we delayed contacting hospitals for block retrieval (Task 1). Once retrieval was underway, we decided to request the archived tumor tissue blocks in waves, in a further effort to prevent the participating hospitals from being overburdened. To date, blocks have been requested from all 33 participating hospitals for 934 of the 1,442 subjects (64.8%). Six of the 33 hospitals (18.2%) have provided us with all requested materials, and blocks have been successfully retrieved for 331 case participants (23.0%).

Tasks 2 and 3. For the laboratory component, services (slide preparation and laboratory assays of p53), have been completed on 90 case women (6.2%) using the following methods. The paraffin blocks from each case participant are used to generate 15- 5 micron and 10-10 micron thick 5-micron slides. Selected sections are baked at 60°C for 30 minutes. Twenty-three sections are banked (protected from light and stored at -20°C) for future molecular studies. Because such little tissue material is needed for the assay and the diagnoses of breast cancer are so recent, few cases are expected to have a tissue sample that is inadequate or too small. However, in no instance is the block exhausted. Instead, because the goal is to return one-half of the archived tumor tissue to the lending institution, the final quantity of slides cut is based on the tissue available. Thus in some instances, less than 25 slides are cut, prepared, and banked.

For the ongoing study, slides are being used to evaluate for evidence of *p53* protein overexpression by immunohistochemical staining utilizing antibodies with high sensitivity for these oncogenes in paraffin-embedded tissues. Because of the recent concern regarding possible degradation of *p53* with time since the paraffin blocks have been cut and fixed, the *p53* assays are being conducted as soon as the slide is cut from the block and prepared. The details of the laboratory methods being used are appended (see Gammon 1999a).

Task 4. We have begun developing the program for the data entry component. The statistical analyses will begin once all blocks have been retrieved, cut, stained, and interpreted.

(7) KEY RESEARCH ACCOMPLISHMENTS

- **Initiation of Tumor Bank.** Our research efforts include initiation of a bank of archived tumor issue among a population-based sample of breast cancer cases who were residents of Long Island and who were diagnosed with a first primary invasive or in situ breast cancer between August 1, 1996, and July 31, 1997, and who participated in a comprehensive case-control interview, donated a 40 ml blood sample, and a casual urine sample. The study also included interviews and donation of blood and urine from a population-based sample of control women from the same geographic area. Because there were no age restrictions for eligibility in the parent case-control study, this newly initiated tissue bank may yield one of the largest archived banks based on a population-based study of older women. Thus, this archived tumor bank will be extremely valuable because of its unique link to risk factor data as well as results of laboratory assays based on other types of biologic specimens, and the unusual age range of the study subjects.

(8) REPORTABLE OUTCOMES

- **Publications.** Two manuscripts, listed below and included in the appendix, have been published from a previous ARMY award (DAMD94-j-4250), and are being used **to guide the research protocol for the ongoing project**. Data from these projects are based on breast cancer cases who were under age 45 years at diagnosis and residents of New Jersey state.

  (1) **Gammon MD, Hibshoosh H**, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Cigarette smoking and other risk factors in relation to *p*53 protein expression in breast cancer among young women. Cancer Epidemiology, Biomarkers and Prevention 1999;8:255-263.

  (2) **Gammon MD, Hibshoosh H**, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Oral contraceptive use and other risk factors in relation to HER-2/*neu* overexpression in breast cancer among young women. Cancer Epidemiology, Biomarkers and Prevention 1999;8:413-419.

- **Presentations.** Presentations made by the Principal Investigator over the past year in which the design and conduct of the ongoing study were discussed are listed below.

1999 "Breast Cancer and the Environment: What is the Evidence for a Link?" Division of Research, Kaiser Permanente, Oakland, CA, April

"Breast Cancer and the Environment: What is the Evidence for a Link?" University of North Carolina, Department of Epidemiology, Chapel Hill, NC, March

"Long Island Breast Cancer Study Project," External Advisory Committee, National Institute of Environmental Health Sciences Center, Environmental Health in Northern Manhattan, Columbia University, New York, NY, February

"Environmental Risk Factors and p53 Expression in Breast Cancer," Department of Surgery, New York Presbyterian Medical Center, New York, NY, February

"Breast Cancer and Environmental Risk Factors: What is the Evidence for a Link?" Mt. Sinai School of Medicine, Derald Ruttenberg Cancer Center, New York, NY, January

"Breast Cancer and the Environment: What is the Evidence for a Link?" Department of Epidemiology Seminar Series, School of Public Health, University of Minnesota, Minneapolis, MN, January

1998 "Long Island Breast Cancer Study Project," Breast Cancer Clusters Workshop sponsored by the U.S. Public Health Service's Office on Women's Health, Boston, MA, December

"Cigarette Smoking and Other Risk Factors in Relation to p53 Expression in Breast Cancer," Memorial Sloan-Kettering Cancer Center, Cancer and Public Health Seminar Series, Department of Epidemiology and Biostatistics/Department of Psychiatry, New York, NY, November

"Environmental Risk Factors and p53 Expression in Breast Cancer," Breast Cancer Research Program, New York-Presbyterian Medical Center, New York, NY, November

(9) CONCLUSIONS

The goal of the ongoing molecular epidemiology component of the Long Island Breast Cancer Study Project are to evaluate the relation between p53 expression measured by immunohistochemistry and environmental risk factors for breast cancer. This project is at the completion of the first year of three-years of funding from the U.S. Army. All four components of the study protocol (block retrieval, slide preparation, lab assays, and data entry) have been initiated as planned. Although implementation of the field activities was delayed, due to unforseen delays in the NIH-funded parent study on which this ongoing study is based, research efforts are underway and close to the target goals. No unusual problems in

any of the four study components have been encountered to date. Two manuscripts have been published by the principal investigator based on results from a previous ARMY-funded project conducted among women in New Jersey, and are being used to guide the ongoing research efforts.

(10) REFERENCES

None.

(11) APPENDICES

Publications. Two manuscripts, listed below and included in the appendix, have been published that are based on results from a previous ARMY award (DAMD94-j-4250). Both are being used **to guide the research protocol for the ongoing project.**

(1) **Gammon MD, Hibshoosh H**, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Cigarette smoking and other risk factors in relation to *p*53 protein expression in breast cancer among young women. Cancer Epidemiology, Biomarkers and Prevention 1999a;8:255-263.

(2) **Gammon MD, Hibshoosh H**, Terry MB, Bose S, Schoenberg JB, Brinton LA, Bernstein JL, Thompson WD. Oral contraceptive use and other risk factors in relation to HER-2/*neu* overexpression in breast cancer among young women. Cancer Epidemiology, Biomarkers and Prevention 1999b;8:413-419.

(12) BINDING

This year 1 report has been bound as directed, using staples in the upper left had corner.

(13) FINAL REPORTS

Not applicable.

Cigarette Smoking and Other Risk Factors in Relation to p53 Expression in Breast Cancer among Young Women[1]

Marilie D. Gammon,[2] Hanina Hibshoosh, Mary Beth Terry, Shikha Bose, Janet B. Schoenberg, Louise A. Brinton, Jonine L. Bernstein, and W. Douglas Thompson

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Abstract

p53 mutations may be a fingerprint for cigarette smoking and other environmental carcinogens, including breast carcinogens. This study was undertaken to explore whether p53 mutations are associated with environmental or other suspected or established risk factors for breast cancer. p53 protein detection by immunohistochemistry (which is more easily quantified in large epidemiological studies than are mutations, and are highly correlated with them) was determined for 378 patients from a case-control study of breast cancer. In this population-based sample of women under the age of 45 years, 44.4% (168/378) of the cases had p53 protein detected by immunohistochemistry (p53+). Polytomous logistic regression was used to calculate the odds ratios (ORs) for p53+ and p53− breast cancer, as compared with the controls, in relation to cigarette smoking and other factors. The ratio of the ORs was used as an indicator of heterogeneity in risk for p53+ *versus* p53− cancer. The ratio of the ORs in a multivariate model was substantially elevated among women with a greater than high school education [2.39; 95% confidence interval (CI), 1.43–4.00], current cigarette smokers (1.96; 95% CI, 1.10–3.52), and users of electric blankets, water beds, or mattresses (1.78; 95% CI, 1.11–2.86). Nonsignificant heterogeneity was noted for family history of breast cancer and ethnicity but not for other known or suspected risk factors. Coupled with the strong biological plausibility of the association, our data support the hypothesis that in breast cancer, as with other tumors, p53 protein immunohistochemical detection may be associated with exposure to environmental carcinogens such as cigarette smoking.

Introduction

Mutations in the *p53* tumor suppressor gene have been implicated in almost all cancer cell types arising from a wide spectrum of tissues and are seen in ~15–50% of breast cancer (1). The functions of the *p53* gene are diverse, including DNA binding, cell cycle control, DNA repair, differentiation, genomic plasticity, and apoptosis (2, 3). Specific *p53* mutations, known as signatures or fingerprints, have been shown to be correlated with environmental exposures, revealing important clues for disease etiology (3, 4). For example, much research has focused on aflatoxin exposure and its correlation with G→T transversion at the third bp of codon 249 in tumor tissue from liver cancer cases (5). Associations with specific *p53* mutations have also been found for sunlight exposure and skin carcinoma, cigarette smoke and lung cancer, tobacco and alcohol and head and neck carcinoma, and vinyl chloride and hepatic angiosarcoma (3, 6). Although little is known about specific fingerprints in the *p53* gene for breast cancer, the mutational spectrum in the *p53* gene of breast cancer cases resembles the pattern of lung cancer mutations, which may likely be related to environmental factors such as cigarette smoking; ~20% of *p53* mutations in breast cancer are G→T transversions, characteristic of bulky carcinogens (7, 8).

Epidemiological research to illuminate broad patterns between risk factors and p53 protein expression detected by immunohistochemistry is a first step and key link in the process of identifying such fingerprints for environmental exposures. *p53* can be measured directly through mutational analysis of chromosomal changes or indirectly through abnormalities in the protein product. Measurement of expression of the protein product through immunohistochemistry is more feasible for large-scale epidemiological research. Much data exist to suggest a strong correlation between p53 protein immunohistochemical expression and mutation (9, 10). After associations are found between epidemiological risk factors and p53 protein expression, direct mutational analysis could then be examined with respect to these environmental exposures.

Tumor markers have been used mainly to subdivide cases for prognostic purposes. More recently, researchers have also used markers for etiological investigations. Tumor markers may help define more homogeneous case groups, yielding clearer patterns with risk factors. For example, such methods proved fruitful in examining the risk of acute myeloid leukemia and various occupational exposures (11). This study was undertaken to examine the role of p53 protein expression, assessed by immunohistochemistry, in breast cancer in relation to

Received 8/27/98; revised 12/7/98; accepted 1/4/99.

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[1] This research was supported in part by Grant DAMD-94-j-4250 from the United States Army Medical Research and Material Command and Grant 1R21CA/ES66224 from the National Cancer Institute and the National Institute of Environmental Health Sciences.

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Table 1 Characteristics of breast cancer cases with available tumor tissues *versus* breast cancer cases without available tissue among young women under the age of 45 years in New Jersey, 1990–1992

	Cases with available tissue (n = 401)	Cases without available tissue (n = 108)	P
Age at diagnosis			
23–29 years	3.5	1.8	0.55
30–34 years	14.7	13.0	
35–39 years	28.2	34.3	
40–44 years	53.6	50.9	
Stage at diagnosis (%)			
In situ	12.4	12.1	0.96
Local	49.9	51.4	
Regional/Distant	37.7	36.5	
ER status (%)			
No test or unknown	15.2	17.6	0.81
Positive	44.9	47.2	
Borderline	8.2	6.5	
Negative	31.7	28.7	
PR status (%)			
No test or unknown	17.2	21.3	0.23
Positive	48.6	47.2	
Borderline	5.0	0.9	
Negative	29.2	30.6	
Race (%)			
White	85.0	85.2	0.74
Black	10.0	8.3	
Asian and other	5.0	6.5	
Education (%)			
HS/Tech[a]	34.2	31.5	0.66
Some college	24.4	22.2	
College graduate	41.4	46.3	
Religion (%)			
Protestant	31.9	35.2	0.23
Jewish	10.5	12.9	
Catholic	54.4	45.4	
Other/None	3.2	6.5	
OC use (%)			
Never	33.2	35.2	0.69
Ever	66.8	64.8	
Age at first full-term birth (%)			
Nulliparous	21.4	19.4	0.58
14–19 years	9.0	11.1	
20–24 years	22.2	21.3	
25–29 years	29.2	24.1	
30+ years	18.2	24.1	
Number of births (%)			
0 births	21.5	19.4	0.38
1 births	18.7	21.3	
2 births	38.4	44.5	
3 or more births	21.4	14.8	
Months of lactation (% among parous)			
None	51.0	54.7	0.54
1+	49.0	45.3	
Number of spontaneous abortions (% among gravid)			
0	75.2	76.8	0.74
1+	24.8	23.2	
Number of induced abortions (% among gravid)			
0	77.5	82.1	0.34
1+	22.5	17.9	
Age at menarche (%)			
8–12 years	56.6	57.4	0.88
13–17 years	43.4	42.6	
Family history of breast cancer (%)			
None	85.0	85.2	0.97
First Degree	15.0	14.8	
Prior breast biopsy (%)			
No	89.5	90.7	0.71
Yes	10.5	9.3	
BMI at interview			
Mean	25.44	24.88	0.30
(SD)	(5.39)	(4.80)	
Physical activity (average 3 time periods)			
Mean	28.36	26.11	0.26
(SD)	(18.28)	(18.23)	
Average caloric intake			
Mean	1593.68	1537.66	0.41
(SD)	(665.17)	(612.73)	
Smoking status (%)			
Never	50.6	51.8	0.82
Ever	49.4	48.2	
Use of alcohol (%)			
None	38.4	38.9	0.39
<7 drinks/week	51.9	55.5	
≥7 drinks/week	9.7	5.6	
Electric blanket and mattress pad use (%)			
Never	64.8	66.7	0.72
Ever	35.2	33.3	

[a] HS/Tech, high school or technical school; BMI, body mass index.

cigarette smoking and other possible and established risk factors.

Materials and Methods

This investigation draws upon the New Jersey subjects from a multicenter, population-based, case-control study (12) that was conducted to determine whether risk for breast cancer among young women was associated with long-term oral contraceptive use, adolescent diet, lifetime alcohol use, and other suspected risk factors for the disease. The 70-min questionnaire was administered by trained interviewers and included assessment of each respondent's family history of breast cancer, reproductive history, menstrual history, contraceptive history, adolescent dietary intake, alcohol consumption, cigarette smoking, body size, physical activity, and other lifestyle factors. At the completion of the main questionnaire, selected anthropometric measures were obtained, and subjects completed a self-administered food frequency questionnaire. Elevated ORs[3] for breast cancer were observed among women who were oral contraceptive users, reported their race as black, consumed higher amounts of alcohol, were not current cigarette smokers, had a low body mass, had a first-degree relative with breast cancer, had a previous breast biopsy, had a late age at first birth, had an early age at menarche, had few or no children, and never breast fed (12–16).

In the New Jersey component of the parent study, cases were women newly diagnosed with *in situ* or invasive breast cancer between May 1, 1990, and December 31, 1992, under the age of 45 years, and residents of one of five centrally

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; OC, oral contraceptive; ER, estrogen receptor; PR, progesterone receptor.

located counties. Controls were identified by random digit dialing (17) in the same five counties as the cases and frequency matched to the anticipated distribution of cases by 5-year age group. In-person interviews were completed with 509 cases (83.4% of eligible women) and 462 controls (76.9%).

For the present study, paraffin-embedded tumor tissue blocks were obtained from the 39 of the 43 hospitals in the New Jersey catchment area where the cases were diagnosed and treated. Blocks were successfully retrieved for 401 (78.8%) of the 509 interviewed cases in New Jersey. As shown in Table 1, the distribution of known and suspected risk factors for breast cancer did not vary significantly between cases with and without tumor tissue available for immunohistochemistry.

The 401 cases with available tissue were evaluated for evidence of p53 protein expression by immunohistochemical staining (18, 19) using an antibody with high sensitivity in paraffin-embedded tissues. Briefly, 5-μm formalin-fixed, paraffin-embedded tissue sections were placed on silane-coated slides and baked at 60°C for 30 min, deparaffinized, hydrated, placed in 10 mM citrate buffer (pH 6), and microwaved for a total of 10 min (antigen retrieval). Appropriate blocking serum (horse serum) and p53 mouse monoclonal antibody clone D01 1:5 dilution (Immunotech, Inc., Westbrook, ME) were used. The detection method used the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). The chromogen diaminobenzidine was used, and sections were counterstained with methyl green (ethyl green; Sigma Chemical Co., St. Louis, MO).

Nuclear staining of tumor and normal tissue, from a single slide, was evaluated by a semiquantitative scoring system for intensity and percentage of positive nuclei. The system assesses the nuclear staining intensity as a 4-level ordered categorical variable (0, none; 1, mild; 2, moderate; and 3, strong), and the percentage of positive cells are assessed as a 5-level ordered categorical variable (0, none or rare cells; 1, <10%; 2, 10–25%; 3, 25–50%; and 4, >50%). Case tumors were considered positive if the nuclear immunohistochemical staining to detect expression of p53 protein had an intensity score of moderate or strong, had at least 10% or more of cells showing evidence of expression, and was considered positive by both study pathologists (H. H, S. B.). The rationale for the cutoff point was based on the background level of the normal adjacent breast tissue on the tumor sections; tumor tissue that showed staining below this threshold was considered negative for p53 protein expression by immunohistochemical detection. Appropriate positive and negative (staining lacking primary antibodies) controls were used in each batch of staining.

Unordered polytomous logistic regression (20) was used to calculate the ORs and 95% CIs for p53-positive (p53+) breast cancer and p53-negative (p53−) breast cancer, as compared with the controls, in relation to cigarette smoking, OC use, age at menarche, age at first birth, parity, lactation, induced abortion, family history of breast cancer, previous breast biopsy, body size, usual alcohol use, race, education, electric blanket use, physical activity, caloric intake, intake of vegetables and fruit, and consumption of fat adjusted for calories (21). To formally test for heterogeneity in the the ORs for p53+ *versus* p53− breast cancer, the ratio of the ORs and the corresponding confidence interval were computed. Best fitting models were developed from a saturated model, including all known and suspected risk factors for breast cancer assessed in the parent study (see Table 1; 12, 16) and then excluding covariates that did not improve the overall fit of the model, as measured by the log likelihood ratio test (20).

Polytomous logistic regression (20) was also used to examine whether risk factor estimates varied among the p53+ cases or the p53− cases categorized by stage of disease (local + *in situ*/regional + distant) or ER status (ER+/ER−; with unknown and borderline excluded due to small numbers).

Table 2 Tumor characteristics and selected demographic factors of breast cancer cases by p53 status and controls among young women under the age of 45 years in New Jersey, 1990–1992

	p53+ (n = 168)	p53− (n = 210)	Controls (n = 462)	P
Age at diagnosis				
23–29 years	4 (2.4%)	9 (4.3%)	27 (5.8%)	0.16
30–34 years	28 (16.7%)	29 (13.8%)	83 (18.0%)	
35–39 years	51 (30.4%)	57 (27.1%)	147 (31.8%)	
40–44 years	85 (50.6%)	115 (54.8%)	205 (44.4%)	
Stage at diagnosis (%)				
In situ	13 (8.0%)	31 (14.8%)		0.24
Local	83 (50.9%)	100 (47.9%)		
Regional/Distant	67 (41.1%)	78 (37.3%)		
ER status (%)				
No test or unknown	20 (11.9%)	34 (16.2%)		0.07
Positive	72 (42.9%)	99 (47.1%)		
Borderline	11 (6.5%)	21 (10.0%)		
Negative	65 (38.7%)	56 (26.7%)		
PR status (%)				
No test or unknown	23 (13.7%)	38 (18.1%)		0.70
Positive	85 (50.6%)	98 (46.7%)		
Borderline	9 (5.4%)	11 (5.2%)		
Negative	51 (30.4%)	63 (30.0%)		
Race (%)				
White	135 (80.4%)	185 (88.1%)	382 (82.7%)	0.23
Black	21 (12.5%)	18 (8.6%)	48 (10.4%)	
Asian and other	12 (7.1%)	7 (3.3%)	32 (6.9%)	
Religion (%)				
Protestant	58 (34.5%)	66 (31.4%)	154 (33.3%)	0.71
Jewish	18 (10.7%)	21 (10.0%)	46 (10.0%)	
Catholic	85 (50.6%)	118 (56.2%)	238 (51.5%)	
Other/None	7 (4.2%)	5 (2.4%)	24 (5.2%)	

Results

The prevalence of p53 protein expression detected by immunohistochemistry in the archival tumor tissue was successfully determined for 378 cases (94.3% of available tissue). p53 expression could not be determined from the tumor tissue of the remaining 5.7% of cases, mainly due to the lack of sufficient tumor tissue in the archived block that was retrieved. In this population-based sample, 44.4% (168/378) of the cases showed evidence of p53 protein detected by immunohistochemistry.

Table 2 shows the distribution of clinical characteristics and selected demographic factors among this population-based sample of p53+ cases, p53− cases, and controls. Prevalence of p53 expression by immunohistochemistry did not increase with age among this sample of younger women. Similarly, there was little variation in the distribution of p53 expression with religion. Although the prevalence was higher among black (53.9%) or Asian and other (63.2%) case women than among white cases (42.2%), the differences were not statistically significant. The prevalence of p53 expression was lower in women diagnosed with *in situ* disease (29.6%) than those with local (45.4%) or regional/distant (46.2%) invasive cancer. In addition, p53 positivity was more common among women with tumors that were ER negative (ER−, 53.7%) than among those with ER-positive tumors (ER+, 42.1%), although there was no variation with PR status.

Table 3 shows the age-adjusted ORs for breast cancer in

Table 3 Age-adjusted ORs and 95% CIs for p53+ and p53− breast cancer in relation to known and suspected breast cancer risk factors among women under the age of 45 years in New Jersey, 1990–1992

	Controls (n = 462)	p53+ cases (n = 168)	p53− cases (n = 210)	p53+ age-adjusted OR (95% CI)	p53− age-adjusted OR (95% CI)	Ratio of the ORs (95% CI)
Environmental factors						
Cigarette smoking						
Never	248	81	109	1.0	1.0	
Former	100	44	58	1.33 (0.86–2.06)	1.30 (0.88–1.94)	1.02 (0.63–1.66)
Current	113	43	43	1.18 (0.77–1.83)	0.87 (0.57–1.33)	1.36 (0.81–2.26)
Alcohol use (drinks/week)						
None	197	72	73	1.0	1.0	
<7	227	77	119	0.93 (0.64–1.35)	1.41 (1.00–2.00)	0.66 (0.43–1.01)
≥7	38	19	18	1.38 (0.75–2.56)	1.30 (0.70–2.44)	1.06 (0.51–2.18)
Electric blanket and mattress pad use						
Never	325	100	146	1.0	1.0	
Ever	137	68	64	1.59 (1.10–2.30)	1.02 (0.72–1.47)	1.55 (1.01–2.38)
Electric blanket and mattress pad use (in months)						
Never	325	100	146	1.0	1.0	
1–9	41	23	18	1.79 (1.03–3.14)	0.96 (0.53–1.73)	1.87 (0.96–3.65)
10–29	46	20	24	1.41 (0.79–2.49)	1.16 (0.68–1.97)	1.21 (0.64–2.32)
≥30	50	25	22	1.60 (0.94–2.72)	0.96 (0.56–1.65)	1.66 (0.89–3.11)
Reproductive factors						
OC use						
Never–<0.5 year	168	55	71	1.0	1.0	
0.5–5 years	176	66	86	1.13 (0.75–1.72)	1.14 (0.78–1.67)	1.00 (0.62–1.60)
5–9 years	81	30	34	1.14 (0.68–1.92)	1.02 (0.62–1.66)	1.12 (0.61–2.05)
≥10 years	37	17	19	1.47 (0.76–2.82)	1.27 (0.68–2.37)	1.15 (0.55–2.42)
Parity						
Ever parous	361	133	164	1.0	1.0	
Never parous	101	35	46	1.03 (0.66–1.61)	1.15 (0.77–1.73)	0.89 (0.54–1.49)
Age at first birth (each additional year)				1.03 (0.99–1.07)	1.04 (1.00–1.08)	0.99 (0.95–1.04)
Children (among parous women)						
1	92	33	38	1.0	1.0	
2	161	64	80	1.08 (0.66–1.77)	1.17 (0.74–1.87)	0.92 (0.52–1.64)
≥3	108	36	46	0.89 (0.51–1.56)	0.98 (0.58–1.65)	0.91 (0.48–1.74)
Lactation (among parous women)						
Never	179	71	79	1.0	1.0	
Ever	177	61	85	0.95 (0.63–1.43)	1.24 (0.85–1.81)	0.77 (0.48–1.23)
Induced abortion (among gravid women)						
Never	305	115	137	1.0	1.0	
Ever	100	37	38	1.05 (0.68–1.61)	0.81 (0.54–1.24)	1.29 (0.78–2.14)
Age at menarche (yr)						
8–12	230	86	127	1.0	1.0	
≥13	232	82	83	0.93 (0.65–1.32)	0.65 (0.46–0.90)	1.43 (0.95–2.17)
Energy balance						
Body Size (BMI)[a]						
<23	144	66	81	1.0	1.0	
23–26	149	53	55	0.79 (0.51–1.21)	0.67 (0.44–1.01)	1.18 (0.72–1.94)
≥27	142	43	71	0.66 (0.42–1.03)	0.88 (0.59–1.31)	0.74 (0.45–1.22)
Physical activity (average of three time periods, relative units in quartiles)						
1 (low)	113	43	47	1.0	1.0	
2	119	43	54	0.98 (0.60–1.62)	1.15 (0.72–1.84)	0.86 (0.48–1.53)
3	115	38	54	0.91 (0.55–1.52)	1.20 (0.75–1.93)	0.76 (0.42–1.37)
4 (high)	115	44	55	1.06 (0.64–1.74)	1.23 (0.77–1.98)	0.86 (0.48–1.53)
Caloric intake (Kcal, in quartiles)						
<1100	112	33	41	1.0	1.0	
1100–1450	113	37	66	1.18 (0.69–2.02)	1.69 (1.06–2.73)	0.69 (0.38–1.28)
1450–1830	112	41	41	1.32 (0.78–2.25)	1.07 (0.64–1.79)	1.23 (0.65–2.32)
≥1830	112	52	57	1.71 (1.02–2.87)	1.54 (0.95–2.50)	1.11 (0.61–2.02)
Dietary fat[b] intake (grams, in quartiles)						
<43.9	114	39	56	1.0	1.0	
43.9–<58.2	111	34	51	0.74 (0.40–1.41)	0.68 (0.39–1.18)	1.11 (0.55–2.24)
58.2–<79.1	112	38	42	0.73 (0.35–1.52)	0.56 (0.29–1.07)	1.31 (0.58–2.97)
≥79.1	112	52	56	0.88 (0.37–2.09)	0.70 (0.31–1.56)	1.26 (0.47–3.35)
Fruit consumption[b] (average weekly servings, in quartiles)						
<2.1	128	42	48	1.0	1.0	
2.1–<4.9	125	34	54	0.84 (0.50–1.42)	1.25 (0.78–1.99)	0.68 (0.37–1.23)
4.9–<9.1	93	47	44	1.47 (0.89–2.42)	1.24 (0.75–2.02)	1.19 (0.66–2.14)
≥9.1	103	40	59	1.13 (0.68–1.90)	1.52 (0.95–2.43)	0.75 (0.42–1.34)
Vegetable consumption[b] (average weekly servings, in quartiles)						
<9.1	125	37	52	1.0	1.0	
9.1–<13.3	113	40	47	1.11 (0.66–1.86)	0.95 (0.59–1.53)	1.16 (0.64–2.13)
13.3–<19.6	107	47	48	1.34 (0.80–2.25)	0.99 (0.61–1.62)	1.35 (0.74–2.45)
≥19.6	104	39	58	1.08 (0.63–1.86)	1.22 (0.76–1.96)	0.89 (0.48–1.63)
Other factors						
Family history of breast cancer						
None	431	137	183	1.0	1.0	
First degree	31	31	27	3.05 (1.78–5.21)	1.94 (1.12–3.35)	1.57 (0.89–2.76)

Table 3 Continued

	Controls (n = 462)	p53+ cases (n = 168)	p53− cases (n = 210)	p53− age-adjusted OR (95% CI)	p53− age-adjusted OR (95% CI)	Ratio of the ORs (95% CI)
Previous biopsy						
None	440	152	186	1.0	1.0	
≥1	22	16	24	2.00 (1.02–3.92)	2.48 (1.35–4.55)	0.81 (0.41–1.58)
Education						
HS/Tech	160	40	83	1.0	1.0	
Some college	116	55	42	1.96 (1.22–3.15)	0.72 (0.46–1.13)	2.71 (1.56–4.70)
College graduate	186	73	85	1.65 (1.06–2.58)	0.92 (0.63–1.33)	1.80 (1.10–2.95)
Race						
Whites	382	135	185	1.0	1.0	
Blacks	48	21	18	1.24 (0.72–2.16)	0.77 (0.44–1.37)	1.61 (0.83–3.15)
Asian/Other	32	12	7	1.08 (0.54–2.16)	0.47 (0.20–1.08)	2.30 (0.88–6.01)

[a] BMI, body mass index; HS/Tech, high school or technical school.
[b] Dietary variables adjusted for both age and caloric intake.

relation to established and suspected risk factors with the breast cancer cases categorized by p53 immunohistochemical detection. The ratio of the ORs was statistically significant in relation to education (OR, 2.67 for greater than high school education; CI, 1.54–4.64) and electric blanket use (1.55; 95% CI, 1.01–2.38). In these age-adjusted analyses, there was no substantial heterogeneity in the ratio of the ORs for the other known and suspected risk factors listed in Table 3, including OC use, lactation, religion, number of births, number of induced or spontaneous abortions, age at menarche, physical activity, fat consumption, and fruit or vegetable intake.

Table 4 shows the factors that were significantly associated with p53+ reast cancer, p53− breast cancer, or displayed significant heterogeneity in the ratio of the OR in a multivariate model. The ratios of the ORs in this model were substantially elevated among women with a greater than high school education (2.39; 95% CI, 1.43–4.00), current cigarette smokers (1.96; 95% CI, 1.10–3.52), and users of electric blankets, water beds, or mattresses (1.78; 95% CI, 1.11–2.86).

The elevated OR for p53+ breast cancer among women with a mother or sister with a history of breast cancer (2.86; 95% CI, 1.61–5.08) was higher than the corresponding ORs among women with p53− breast cancer (1.70; 95% CI, 0.95–3.04). As shown in Table 4, this difference in the ORs for each of the two types, however, was not statistically significant (ratio of the ORs, 1.69; 95% CI, 0.92–3.09). In addition, the OR was elevated for p53+ breast cancer among black women (1.65; 95% CI, 0.88–3.10), whereas the OR was reduced for p53− breast cancer among blacks (0.80; 95% CI, 0.42–1.54). The 2-fold increase in the ratio of the ORs, however, was not statistically significant (95% CI, 0.96–4.43). Other factors that were found to affect breast cancer risk in these data, such as caloric intake, did not vary with p53 status (see Table 4).

In Table 5, the ratio of the ORs for p53+ breast cancer and p53− cancer in relation to other patterns of cigarette smoking did not vary substantially from the ratio of the ORs for smoking shown in Table 4. For example, the ratio of the ORs derived from multivariate-adjusted models were elevated among women who were heavy smokers (1.66 for 16+ pack-years; 95% CI, 0.86–3.18) and among those who began smoking before age 16 years (1.81; 95% CI, 0.81–4.04); this heterogeneity is very similar to the corresponding heterogeneity observed for current smoking, as shown in Table 4.

The heterogeneity in risk noted with electric blanket use appeared to be restricted to women who used the devices continuously throughout the night (ratio of the OR, 1.98; 95% CI, 1.20–3.26) and not among those who used the device to warm the bed only (corresponding ratio of the OR, 1.06; 95% CI, 0.41–2.76).

The p53+ cases and the p53− cases were further categorized by stage of disease. The OR in relation to current cigarette smoking for women with local and *in situ* disease was 1.50 for p53+ breast cancer and 0.65 for p53− breast cancer. The ratio of the OR was 2.29 (95% CI, 1.08–4.84). The OR for current smoking for women with regional and distant stage disease was 1.03 for p53+ cancer and 0.57 for p53− cancer. The ratio of the OR was 1.82 (95% CI, 0.68–4.85).

With further categorization by ER status, the OR in relation to current smoking for women with ER+ breast cancer was 1.99 for p53+ disease and 0.62 for p53− disease; the ratio of the OR was 3.21 (95% CI, 1.31–7.87). The corresponding OR for current smoking for women with ER− breast cancer was 0.91 for p53+ disease and 0.57 for p53− disease; the ratio of the OR was 1.60 (95% CI, 0.54–4.78). Categorization by ER status showed no heterogeneity in the ratio of the ORs for electric blanket use (data not shown).

Discussion

This study is based on immunohistochemical detection of p53 protein expression in a large, population-based series of archived tumor tissue of 378 breast cancer patients who were diagnosed between 1990 and 1992 in 39 hospitals in a five-county area in central New Jersey. The laboratory results on p53 expression were coupled with risk factor data collected as part of a case-control study conducted previously (12, 16). Possible limitations to our study that may affect interpretation of our results include the multiple comparisons made during our statistical analyses. Although many known and suspected risk factors were examined, heterogeneity was primarily observed with environmental factors, or a possible surrogate marker for such exposures, adding more credence to our results. Another potential disadvantage to consider is that the power to assess possible variation among subgroups of cases in our study was limited. A larger sample size would have permitted a more thorough exploration of possible etiological heterogeneity by p53 status.

Determination of specific p53 mutations would have resulted in less misclassification of p53 status than detection of p53 protein expression by immunohistochemistry, as was done in the study reported here. Although the overall prevalence of immunohistochemical detection may be higher than the prevalence of mutations (and both false-negatives as well as false-positives are possible; 9, 22), data exist to suggest a strong

Table 4 Multivariate adjusted[a] ORs and 95% CIs for p53+ and p53− breast cancer among women under the age of 45 years in New Jersey, 1990–1992

	p53+ OR (95% CI)	p53− OR (95% CI)	Ratio of the ORs (95% CI)
Race			
White	1.0	1.0	
Black	1.65 (0.88–3.10)	0.80 (0.42–1.54)	2.06 (0.96–4.43)
Asian/Other	1.09 (0.49–2.43)	0.60 (0.25–1.47)	1.81 (0.64–5.11)
Education			
High school	1.0	1.0	
Any college	1.66 (1.05–2.64)	0.69 (0.47–1.03)	2.39 (1.43–4.00)
Alcohol use (drinks/week)			
None	1.0	1.0	
<7	0.65 (0.42–1.00)	1.27 (0.85–1.88)	0.51 (0.31–0.84)
7+	1.11 (0.56–2.22)	1.03 (0.51–2.13)	1.07 (0.48–2.43)
Body mass index			
<23	1.0	1.0	
23–26	0.83 (0.53–1.32)	0.68 (0.44–1.06)	1.22 (0.72–2.06)
27+	0.63 (0.39–1.04)	0.84 (0.55–1.30)	0.75 (0.44–1.30)
Age at first birth (for each additional year)	1.02 (0.97–1.07)	1.05 (1.00–1.09)	0.98 (0.93–1.03)
Parity status			
Ever	1.0	1.0	
Never	0.99 (0.60–1.63)	1.26 (0.80–1.97)	0.79 (0.45–1.38)
Age at menarche			
8–12	1.0	1.0	
13+	0.75 (0.51–1.11)	0.60 (0.42–0.85)	1.26 (0.81–1.96)
Family history			
None	1.0	1.0	
First degree	2.86 (1.61–5.08)	1.70 (0.95–3.04)	1.69 (0.92–3.09)
Prior breast biopsy			
No	1.0	1.0	
Yes	1.82 (0.84–3.94)	3.16 (1.62–6.17)	0.58 (0.27–1.23)
Caloric intake (Kcal, in quartiles)			
<1100	1.0	1.0	
1100–1450	1.32 (0.75–2.33)	1.68 (1.03–2.76)	0.78 (0.41–1.49)
1450–1830	1.34 (0.75–2.38)	1.07 (0.63–1.84)	1.25 (0.63–2.45)
≥1830	1.98 (1.14–3.44)	1.71 (1.02–2.86)	1.16 (0.61–2.18)
Electric blanket and mattress pad use			
Never	1.0	1.0	
Ever	1.56 (1.04–2.35)	0.87 (0.59–1.29)	1.78 (1.11–2.86)
Cigarette smoking			
Never	1.0	1.0	
Former	1.66 (1.02–2.70)	1.18 (0.77–1.84)	1.40 (0.82–2.39)
Current	1.29 (0.79–2.11)	0.66 (0.41–1.06)	1.96 (1.10–3.52)

[a] Adjusted for all other variables in the table.

correlation between p53 protein expression and mutations (9, 10, 23). Because of the difficulty of determining specific mutations in a large-scale epidemiological study such as ours, detection of protein expression by immunohistochemistry first could help narrow the search for mutations. Thus, the study reported here should be viewed as a first step in evaluating the link between cigarette smoking, p53 status, and breast cancer risk.

There is also the possibility that lack of consideration in the storage and handling in the preparation of archived tissue for immunohistochemistry results in attenuation of the estimated prevalence of p53 expression (24), although this has not been confirmed by others (25). Our laboratory methods were undertaken prior to these published reports, and the length of time between cutting, staining, and immunohistochemical evaluation was not recorded. However, it is reassuring that the 44% prevalence in p53 expression observed in our case series is comparable with that reported by others (1, 26).

Results from one previous case-control investigation (27) conducted among Dutch women under the age of 55 years are supportive of our observations with a 1.55 unadjusted ratio of the ORs for p53+ *versus* p53− breast cancer in relation to current cigarette smoking. Although the heterogeneity observed in the Dutch study was not statistically significant, their results may have been attenuated by possible misclassification of p53 status (28). In the only other study (29) to examine whether breast cancer risk factors varied with p53 status, which was based on a case series of node-negative patients in a major cancer center in New York City, tobacco and other environmental risk factors were not assessed.

The role of cigarette smoking on breast carcinogenesis is unclear. Many epidemiological investigations have found that smoking does not affect breast cancer risk (30–35), including three previous studies that also focused on young women (31, 34, 35). A few other studies (16, 36–39), including ours (16), have found a decrease in risk in relation to current smoking. Others have observed an increase in risk in at least one subgroup of women (32, 40–46). Previous investigators have hypothesized that a potentially carcinogenic effect, as well as a possible antiestrogen effect, of cigarette smoking on breast cancer are biologically plausible (40, 47). Stratification of breast cancer cases by p53 status, or other genetic markers such

Table 5 Patterns of cigarette smoking (multivariate adjusted[a] ORs and 95% CIs) for p53+ and p53− breast cancer among women under the age of 45 years in New Jersey, 1990–1992

	Controls (n = 462)	p53+ cases (n = 168)	p53− cases (n = 210)	p53+ OR (95% CI)	p53− OR (95% CI)	Ratio of the ORs (95% CI)
Among ever smokers						
Duration of smoking (pack-years)						
Never	248	81	109	1.0	1.0	
<5	69	26	38	1.22 (0.69–2.17)	1.04 (0.63–1.71)	1.18 (0.63–2.21)
5–15	73	31	28	1.81 (1.06–3.13)	0.82 (0.48–1.40)	2.23 (1.18–4.22)
≥16	71	30	35	1.41 (0.80–2.48)	0.85 (0.50–1.44)	1.66 (0.86–3.18)
Years of smoking						
Never	248	81	109	1.0	1.0	
<10 years	74	31	28	1.57 (0.91–2.72)	0.73 (0.42–1.25)	2.16 (1.14–4.12)
10–18 years	66	29	39	1.67 (0.95–2.93)	1.43 (0.87–2.36)	1.17 (0.63–2.17)
≥18 years	73	27	34	1.17 (0.66–2.09)	0.69 (0.41–1.17)	1.71 (0.89–3.30)
Number of cigarettes/day						
Never	248	81	109	1.0	1.0	
<10	59	18	30	0.99 (0.52–1.87)	0.94 (0.55–1.61)	1.05 (0.52–2.14)
10–19	48	21	25	1.73 (0.93–3.21)	1.06 (0.59–1.89)	1.63 (0.82–3.26)
≥20	106	48	46	1.65 (1.02–2.69)	0.81 (0.51–1.29)	2.04 (1.16–3.58)
Age started smoking						
Never	248	81	109	1.0	1.0	
8–15 years	66	16	19	0.92 (0.47–1.79)	0.51 (0.27–0.95)	1.81 (0.81–4.04)
16–17 years	55	27	29	1.84 (1.02–3.29)	1.23 (0.71–2.12)	1.49 (0.78–2.85)
≥18 years	92	44	53	1.60 (0.98–2.60)	1.00 (0.64–1.57)	1.60 (0.93–2.77)
Among current smokers only						
Duration of smoking (pack-years)						
Never	248	81	109	1.0	1.0	
<16	55	20	18	1.48 (0.78–2.81)	0.61 (0.32–1.17)	2.40 (1.10–5.24)
≥16	58	23	25	1.15 (0.60–2.22)	0.67 (0.36–1.25)	1.72 (0.80–3.71)
Years of smoking						
Never	248	81	109	1.0	1.0	
<18 years	51	20	16	1.50 (0.75–2.99)	0.74 (0.37–1.47)	2.03 (0.87–4.71)
≥18 years	62	23	27	1.17 (0.62–2.19)	0.58 (0.32–1.06)	2.01 (0.97–4.19)
Number of cigarettes/day						
Never	248	81	109	1.0	1.0	
<20	49	16	16	1.28 (0.65–2.54)	0.55 (0.28–1.08)	2.35 (1.03–5.37)
≥20	64	27	27	1.32 (0.71–2.45)	0.73 (0.40–1.32)	1.81 (0.88–3.76)
Age started smoking						
Never	248	81	109	1.0	1.0	
8–17 years	64	18	23	0.99 (0.50–1.96)	0.68 (0.37–1.25)	1.47 (0.67–3.24)
≥18 years	49	25	20	1.64 (0.88–3.06)	0.61 (0.32–1.17)	2.69 (1.26–5.73)

[a] Adjusted for age, race, education, alcohol use, body mass index, age at first birth, parity status, age at menarche, family history of breast cancer, prior breast biopsy, caloric intake, and electric blanket use.

at *N*-Acetyltransferase 2 (48), has the potential to yield more etiologically homogeneous groups, where the possible dual effects of cigarette smoking on breast cancer risk may become apparent. Our data showed a modest 29% increase in risk for p53+ breast cancer along with a 34% decrease in risk for p53− breast cancer in relation to current cigarette smoking. Although the individual ORs by p53 status were not statistically significant, this heterogeneity of effect was. Heterogeneity of effect for smoking by p53 status was noted in both late-stage and early-stage disease as well as in ER+ and ER− cancers, although the ratio of the OR was more pronounced among ER+ tumors. No other studies have reported on these associations.

The lack of a dose-response effect for current smoking among our p53+ cases, as compared with controls, may indicate that our results were due to chance. However, a possible link between breast cancer stratified by p53 status and cigarette smoking is biologically plausible. p53 mutations are highly prevalent in most tumor sites, the characteristic mutation patterns have been linked to specific exposures, and DNA adducts have been correlated with specific mutations (49). Furthermore, mutations in the *p53* gene are the most common molecular change in human cancer and have been hypothesized to represent a fingerprint for certain environmental exposures (2, 4). Data supporting an association between tobacco consumption and p53 protein expression and/or mutations have been seen in lung, head and neck, oral, and bladder cancer cases (50–54). Subdividing breast cancer cases by p53 protein expression and searching for important patterns in breast cancer risk factors by p53 status could help to narrow the search for specific p53 mutations.

Although the proportion of breast cancer cases that are due to germ-line mutations such as BRCA1 are greater in younger women than in older women (55), environmental risk factors such as alcohol consumption have been found to appreciably affect breast cancer risk in young women (14). In addition, a few recent reports have indicated that p53 mutations occur frequently among women with known BRCA1 or BRCA2 mutations (56, 57). Also, among women with a family history of breast cancer, those with a Jewish heritage have been shown to have a higher risk of breast cancer than women who do not

(58). Although we lacked information on BRCA1/BRCA2 mutations in our study population of younger women, we examined whether the ORs for breast cancer stratified by p53 status varied with religion or with family history of breast cancer. We observed no direct relation with religion, but risk for breast cancer was higher among those with a family history for both p53+ and p53− tumors, and the association was slightly more pronounced for p53+ breast cancer.

Whether a positive association between immunohistochemical detection of p53 in breast cancer and use of electric blankets, mattresses, or heated water beds is biologically plausible is not known at this time. Electromagnetic fields have been shown to influence melatonin production in animals, which in turn has been hypothesized to affect estrogen levels and mammary carcinogenesis (59). In epidemiological studies, however, it is unclear whether exposure to electromagnetic fields is associated with breast cancer risk in women. Conflicting results have emerged from studies assessing occupational exposures (60–63), residential proximity to electromagnetic sources (64–67), or use of electric blankets (68–71). Also, there is no other epidemiological evidence that p53+ breast cancer or other p53+ cancers are associated with exposure to electromagnetic fields.

Biological reasons for the heterogeneity of p53+ *versus* p53− breast cancer with education observed in our data are also not clear. Measures of socioeconomic status, such as education and income, have long been recognized as, but poorly understood, risk factors for breast cancer (72). The variable education can be regarded as a surrogate of other unmeasured or poorly measured socially determined characteristics or exposures, including environmental exposures (73). Which of these other factors, or group of factors, education represents in these data are unknown and should be more fully explored.

Laboratory investigations (6) have noted that mutations in the *p53* gene among women with ovarian cancer resemble those found in breast cancer. Thus, in addition to environmental exposures that may play a role in exogenous mutations of the *p53* gene, other exposures (*e.g.*, estrogen-related factors) may also affect p53 protein expression through endogenous mutations. For example, van der Kooy *et al.* (27) reported an increased risk of p53+ tumors for use of OCs of at least 9 years and a protective effect for lactation of at least 25 weeks for p53+ cases only. Schildkraut *et al.* (74) reported a strong association between p53 protein expression in ovarian cancer cases and number of ovulatory cycles. Specifically, women with more than 235 ovulatory cycles had an increased risk of p53+ tumors than p53− tumors, as opposed to women with fewer ovulatory cycles. The investigators hypothesized that because the majority of p53 mutations seen in ovarian cancer are transition, an increased number of ovulatory cycles will increase cellular turnover and therefore increase the likelihood of endogenous mutations. A role for some breast cancer risk factors that influence levels of estrogen and cellular growth in increasing the rate of endogenous mutations may therefore be possible. In the study reported here, however, no substantial heterogeneity of effect by p53 status was noted for long-term OC use, lactation, or other reproductive and menstrual characteristics.

In sum, this is the first report of statistically significant heterogeneity of cigarette smoking with p53 protein expression immunohistochemically detected in breast cancer. The association is biologically plausible; others (3, 4) have hypothesized that p53 mutations in cancer are a fingerprint of environmental exposures, particularly cigarette smoke. The results reported here require confirmation by others, and identification of the specific p53 mutations involved is an important next step.

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Oral Contraceptive Use and Other Risk Factors in Relation to HER-2/*neu* Overexpression in Breast Cancer Among Young Women[1]

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Abstract

This study was undertaken to explore whether the incidence of breast tumors that overexpress HER-2/*neu* protein product (HER-2/*neu*+) is more strongly associated with oral contraceptives (OCs) and other factors than is the incidence of tumors that do not (HER-2/*neu*−). In a population-based sample of women <45 years, 42.9% (159 of 371) of *in situ* and invasive breast cancer cases were HER-2/*neu*+ as assessed by immunohistochemistry in archived tissue. Polytomous logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for HER-2/*neu*+ and HER-2/*neu*− breast cancer, as compared with 462 population-based controls, in relation to OCs and other factors. The ratio of the ORs (HER-2/*neu*+ *versus* HER-2/*neu*− tumors) was used as an indicator of heterogeneity in risk. There was little heterogeneity in risk for OC use of 6 months or more by HER-2/*neu* status (age-adjusted ratio of ORs, 1.29; 95% CI, 0.83–2.00). Among early pill users (≤18 years of age) heterogeneity was apparent (2.39; 95% CI, 1.08–5.30), which was attenuated in a multivariate model (1.99; 95% CI, 0.87–4.54); among cases with estrogen receptor-negative tumors, heterogeneity increased to 5-fold. For other risk factors, there was no marked heterogeneity between + and − tumors for HER-2/*neu*. In summary, the incidence of breast cancer among younger women in relation to OC use at an early age varied with HER-2/*neu* status, with the odds ratio for + tumors twice that for − tumors.

Introduction

Many epidemiological studies (1) have shown no association between breast cancer and OC[3] use. Some studies, however, have shown a modest <2-fold increase among young women with breast cancer in relation to long-term OC use, recent use, or use at an early age (1–3). Because the modest increase could be due to uncontrolled or poorly controlled confounding, the etiological significance of the association is unclear.

Some investigators have suggested that OCs may be more strongly associated with pathologically distinct subgroups of breast cancer. However, results of previous studies that have examined the association with cases classified by tumor morphology or estrogen receptor status have been inconsistent (4). Molecular studies indicate that oncogenes, such as HER-2/*neu* and others, are involved with breast cancer pathogenesis (5) and possibly with tumor initiation (6). Thus, classification of tumors by oncogene overexpression or amplification may produce etiologically distinct subgroups. This strategy has been used successfully in a study of occupational exposures and *ras* oncogene activation in acute myeloid leukemia (7).

One previous study (8) has explored the possible association between OCs and HER-2/*neu* status. The adjusted OR in relation to use of OCs at age 20 years or younger was significantly increased 7-fold for HER-2/*neu*-positive breast cancer among young Swedish women, as compared with cases with tumors that lacked oncogene amplification. The study, however, was based on very limited numbers. A consistent association between OCs and HER-2/*neu*-positive tumors would indicate that either HER-2/*neu* is the mechanism by which OCs affect breast cancer, or the oncogene is a cofactor that interacts with OCs in producing the disease.

With regard to other risk factors for breast cancer, another study (9) has addressed the possible interaction between reproductive risk factors and alterations in the HER-2/*neu* oncogene in breast cancer. In this report from the Netherlands (9), the OR was significantly increased 4-fold for HER-2/*neu*-positive breast cancer, as compared with controls, in relation to late age at first birth and ever having breastfed; the corresponding ORs for HER-2/*neu*-negative breast cancer were 2-fold and less than unity, respectively. Thus, classification of breast cancer cases by the presence of a molecular alteration, and thus into etiologically distinct subgroups, may also help to clarify these relationships as well.

We undertook a population-based study to address the hypothesis that the incidence of HER-2/*neu*-positive tumors is more strongly associated with OC use than is the incidence of

Received 10/8/98; revised 2/11/99; accepted 3/1/99.

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[1] This research was supported in part by Grant DAMD-94-j-4250 from the U.S. Army Medical Research and Material Command and Grant 1R21CA S66224 from the National Cancer Institute and the National Institute of Environmental Health Sciences.

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[3] The abbreviations used are: OC, oral contraceptive; OR, odds ratio; CI, confidence interval; ER, estrogen receptor.

HER-2/*neu*-negative tumors. The study also explored whether HER-2/*neu*-positive tumors are related to other risk factors for breast cancer.

Materials and Methods

This study included three components: (*a*) collection of archived paraffin-embedded tissue blocks from a population-based sample of white and black breast cancer cases; (*b*) laboratory evaluation for evidence of HER-2/*neu* overexpression in the tumor tissue by immunohistochemistry; and (*c*) combining the laboratory results with risk factor information on the same cases to estimate the ORs for HER-2/*neu*-positive breast cancer in relation to OC use and other established and potential risk factors for breast cancer. This study received approval from the institutional review boards of the participating institutions.

Study Subjects and Risk Factor Information. The source of the cases, controls, and the risk factor information is from the New Jersey component of the parent study, which was a multicenter, population-based, case-control study of breast cancer (3). A woman was eligible as a case if she was newly diagnosed with *in situ* or invasive breast cancer between May 1, 1990, and December 31, 1992; was between the ages of 20 to 44 years at diagnosis; and was a resident of a five-county study area in New Jersey (Middlesex, Monmouth, Morris, Somerset, and Union). Potentially eligible case women were ascertained using rapid reporting; field personnel visited hospitals within the five-county study area (as well as those in adjacent counties) on a monthly basis to review pathology reports to identify eligible cases. Physicians of eligible cases were contacted for approval to contact their patients.

A woman was eligible as a control if she was between the age of 20 to 44 years, was a resident of the same five-county area of central New Jersey as cases during the study period, and had access to a residential telephone. Controls were identified by random digit dialing (10) and frequency matched to the expected distribution of cases by 5-year age group. Physician-approved cases and controls were contacted first by letter and then by telephone to seek permission for the in-person interview. Before each interview, the purpose and content of the study was explained, and the informed consent form was signed.

Interviews were completed with 509 cases (83.4% of eligibles) and 462 controls (76.9%). The in-person interview lasted ~70 min and included ascertainment of OC use (using a reproductive and contraceptive calendar along with pictorial memory aids); menstrual and reproductive histories including pregnancies, lactation, and abortions; lifetime alcohol consumption patterns; adolescent diet; body size and development; physical activity; demographic factors; family history of cancer; and medical history including biopsy-proven benign breast disease and gynecological surgery. After completion of the questionnaire, trained interviewers took anthropometric measures such as skinfold thicknesses, circumference measurements, wrist and elbow width, standing and sitting height, and weight. At the conclusion of the interview, respondents completed a comprehensive self-administered food frequency questionnaire that focused on intake of food items over the past year.

Block Retrieval. For this project, retrieval was attempted from the appropriate hospital pathology departments for a representative paraffin-embedded tumor tissue block for each case participant. For the present study, blocks were successfully retrieved for 401 (78.8%) of the interviewed cases. As reported previously (11), the distribution of known and suspected risk factors for breast cancer did not vary significantly between cases with and without tumor tissue available for immunohistochemistry.

Slide Preparation and Laboratory Analyses. HER-2/*neu* overexpression was evaluated in tissue sections by immunohistochemical staining (12, 13) using antibodies with high sensitivity for HER-2/*neu* in paraffin-embedded tissues. The paraffin blocks were used to generate three 5-μm-thick sections on silane-coated slides. The sections were baked at 60°C for 30 min, deparaffinized in xylene, and hydrated in alcohol and water. One of the sections was stained with H&E. Another was immunohistochemically stained with C-*neu* (Ab-3) mouse monoclonal antibody IgG1 (1:50; Calbiochem, Cambridge MA). The slides were stained using the Ventana ES automated immunostainer (Ventana Medical Systems, Inc., Tucson AZ) and then counterstained using the CAS DNA staining kit, which uses the Feulgen staining (Becton Dickinson, San Jose, CA). The stained DNA was quantified using the CAS200 Image Analyzer. The last of the three sections was used as a negative control for the immunohistochemical staining of C-*neu* and was prepared in identical fashion except that the section lacked the C-*neu* primary antibody. In addition, each batch of staining performed had two controls stained in parallel. This includes a CAS control for DNA content consisting of a cell line of known DNA content. A CAS control cell line of known DNA content and overexpressor of C-*neu* with a known C-*neu* protein content was also used as a control (Becton Dickinson).

The H&E section corresponding to each block was reviewed by the two study pathologists (HH and SB) to confirm the diagnosis of cancer. The corresponding areas were searched for in the C-*neu*-stained sections. Areas of cancer showing predominantly membranous red staining were analyzed by the CAS200, provided that the negative control showed minimal background staining. Using the CAS200 Quantitative Image Analyzer (Becton Dickinson), the C-*neu* protein level was quantitated with the Quantitative Oncogene product program, yielding the average pg protein of C-*neu* per cell.

Levels above 0.1 pg/cell were considered elevated and positive of overexpression. For additional statistical analyses, we also considered an alternative cutpoint for positivity of 0.2 pg. Because results were not substantially different from those based on a cutpoint of 0.1, only the latter are shown.

Statistical Analyses. Unordered polytomous logistic regression (14) was used to calculate the ORs and 95% CIs for HER-2/*neu*-positive (+) breast cancer and HER-2/*neu*-negative (−) breast cancer, as compared with the controls, in relation to use of OCs, patterns of OC use, and other factors including age at menarche, age at first birth, parity, lactation, induced abortion, family history of breast cancer, previous breast biopsy, body size, usual alcohol use, race, education, smoking, electric blanket use, physical activity, and caloric intake. The ratio of the ORs (and corresponding CIs; Ref. 15) was used as an indicator of heterogeneity in risk for tumor-positive *versus* tumor-negative cancer. Best fitting models were developed from a saturated model including all known and suspected risk factors for breast cancer and then excluding covariates that did not improve the overall fit of the model as measured by the log likelihood ratio test (14). Cutoff points for the factors that were assessed as continuous variables were based on the distributions observed among the control subjects, with the exception of OCs and cigarette smoking. For these latter variables, cutoff points were used to be consistent with other previous publications (3, 16) of these two controversial topics.

Results

Prevalence of HER-2/*neu* overexpression in the archival tumor tissue was successfully determined for 371 cases with breast can-

Table 1 Characteristics of breast cancer cases by HER-2/*neu* status and controls among young women <45 years of age in New Jersey, 1990–1992

	HER-2/*neu*− (*n* = 159)	HER-2/*neu*− (*n* = 212)	Controls (*n* = 462)	P[a]
Age at diagnosis				
23–29 years	5 (3.14%)	9 (4.25%)	27 (5.84%)	0.28
30–34 years	25 (15.72%)	31 (14.62%)	83 (17.97%)	
35–39 years	49 (30.82%)	57 (26.89%)	147 (31.82%)	
40–44 years	80 (50.31%)	115 (54.25%)	205 (44.31%)	
Stage at diagnosis (%)				
In situ	11 (6.96%)	27 (13.04%)		0.11
Local	77 (48.73%)	104 (50.24%)		
Regional/Distant	70 (44.30%)	76 (36.71%)		
ER status (%)				**0.02**
Positive	62 (44.29%)	109 (59.89%)		
Borderline	14 (10.00%)	17 (9.34%)		
Negative	64 (45.71%)	56 (30.77%)		
Progesterone receptor status (%)				0.34
Positive	78 (56.52%)	106 (59.89%)		
Borderline	6 (4.34%)	13 (7.34%)		
Negative	54 (39.13%)	58 (32.77%)		
Race (%)				
White	131 (82.39%)	182 (85.85%)	382 (82.68%)	0.27
Black	16 (10.06%)	24 (11.32%)	48 (10.39%)	
Asian and other	12 (7.55%)	6 (2.83%)	32 (6.93%)	
Religion (%)				
Protestant	52 (32.70%)	70 (33.02%)	154 (33.33%)	0.61
Jewish	14 (8.81%)	23 (10.85%)	46 (9.96%)	
Catholic	86 (54.09%)	115 (54.25%)	238 (51.52%)	
Other/None	7 (4.40%)	4 (1.89%)	24 (5.19%)	

[a] *P* for χ^2 test. *Bold*, statistically significant heterogeneity.

cer. The remaining 7.5% could not be determined because of the lack of tumor tissue in the archived block retrieved from the hospital. In this population-based sample, 42.9% (159/371) of the breast cancer cases showed overexpression of HER-2/*neu*. The prevalence of overexpression did not increase with age among this sample of younger case women newly diagnosed with breast cancer (Table 1). Case women with HER-2/*neu*-negative tumors were more likely than women with HER-2/*neu*-positive tumors to have ER+ tumors ($P = 0.02$) and to be diagnosed with *in situ* disease ($P = 0.11$). There was little association between HER-2/*neu* status and progesterone receptor status or race.

Table 2 shows the age-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*− breast cancer in relation to patterns of OC use. There was little heterogeneity in risk for OC use for 6 months or more by HER-2/*neu* status (age adjusted ratio of ORs, 1.29; 95% CI, 0.83–2.00). However, among women who started using the pill at age 18 years or earlier, heterogeneity by HER-2/*neu* status was apparent (2.39; 95% CI, 1.08–5.30). There was little or no heterogeneity of association in relation to duration of OC use, recent use, and recently starting or stopping.

Table 2 also shows the age-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*− breast cancer in relation to reproductive and other risk factors for breast cancer, including family history of breast cancer, body size, alcohol, or cigarette smoking. There was evidence of heterogeneity by HER-2/*neu* status among Asian and other women (ratio of the OR, 2.78; 95% CI, 1.02–7.61); however, the number of Asian and other case and control participants (excluding blacks and whites) in our study was small. There was little or no heterogeneity of effect for other factors examined, including age at first birth (ratio of the OR, 0.96 for each additional year; 95% CI, 0.92–1.01), lactation (ratio of the OR, 0.72 for ever *versus* never; 95% CI, 0.44–1.15), or the other factors listed in Table 2.

In Table 3 are the multivariate-adjusted ORs for breast cancer categorized by HER-2/*neu* status. Table 3 includes a variable for age at first use of OCs along with those variables that contributed to a best fitting model as described in "Materials and Methods." The modest heterogeneity in ORs observed for early pill use was no longer statistically significant in a multivariate model (for age 18 or earlier, the ratio of the ORs, 1.99; 95% CI, 0.87–4.54). As shown in Table 3, for other established and suspected breast cancer risk factors, our analyses did not reveal marked heterogeneity in risk between positive and negative HER-2/*neu* tumors.

In Table 4 are the multivariate-adjusted ORs and corresponding CIs for HER-2/*neu*+ and HER-2/*neu*− breast cancer in relation to patterns of OC use with the breast cancer cases further stratified by the ER status of the tumor. Among case women with ER− tumors, the OR for ever use of OCs was 2.58 (95% CI, 1.31–5.10) among HER-2/neu+ cases and 0.92 (95% CI, 0.49–1.71) among HER-2/*neu*− cases. The ratio of the ORs for ever use of OCs was significantly elevated (2.81; 95% CI, 1.18–6.67). The heterogeneity was particularly pronounced among women with age at first use before age 18 years (ratio of the OR, 5.37; 95% CI, 1.20–24.01) or after age 22 years (ratio of the OR, 5.92; 95% CI, 1.81–19.36). Little or no heterogeneity, in relation to OC use (Table 4), was noted among case women with ER+ tumors. When cases were stratified by progesterone receptor status, which is highly correlated to ER status, a similar but attenuated pattern of effect was observed; due to sparse cells, however, modification by ER/PR status combined could not be evaluated. Heterogeneity by stage of disease was not apparent (data not shown). Also, there was little variation in the incidence of HER-2/*neu*+ and HER-2/*neu*− breast cancer in relation to other estrogen-related risk factors when cases were stratified by estrogen/progesterone receptor status.

Discussion

The proto-oncogene HER-2/*neu* is the human homologue of the rat *neu* oncogene and is mapped on chromosome 17 at q21. It has

Table 2 Age-adjusted ORs and 95% CIs for HER-2/*neu*-positive (+) and HER-2/*neu*-negative (−) breast cancer in relation to known and suspected risk factors among women <45 years of age in New Jersey, 1990–1992

	Controls (n = 462)	HER-2/*neu*+ cases (n = 159)	HER-2/*neu*− cases (n = 212)	Age-adjusted OR (95% CI)		Ratio of the ORs (95% CI)
				HER-2/*neu*+	HER-2/*neu*−	
Oral contraceptives						
OC use						
Never	168	48	76	1.0	1.0	
Ever	294	111	136	1.33 (0.90–1.96)	1.03 (0.73–1.44)	1.29 (0.83–2.00)
OC duration (years)[a]						
<5	37	18	18	1.30 (0.85–2.00)	1.00 (0.69–1.47)	1.30 (0.80–2.11)
5–9	81	27	37	1.19 (0.69–2.05)	1.03 (0.64–1.66)	1.16 (0.63–2.14)
≥10	176	66	81	1.75 (0.91–3.36)	1.13 (0.60–2.13)	1.55 (0.73–3.27)
Age at first use of OCs (in years)[a]						
<18	40	20	13	1.91 (1.01–3.59)	0.80 (0.40–1.59)	**2.39 (1.08–5.30)**
18–21	152	48	81	1.10 (0.70–1.74)	1.18 (0.80–1.73)	0.94 (0.56–1.56)
≥22	102	43	42	1.45 (0.90–2.35)	0.89 (0.56–1.39)	1.64 (0.94–2.87)
Number of years since first use[a]						
<15	86	29	32	1.37 (0.77–2.44)	0.96 (0.56–1.63)	1.43 (0.73–2.81)
15–19	113	39	41	1.21 (0.74–1.97)	0.83 (0.53–1.32)	1.45 (0.81–2.57)
≥20	95	43	63	1.44 (0.86–2.41)	1.28 (0.81–2.00)	1.13 (0.64–1.98)
Number of years since last use[a]						
<1	43	13	19	1.25 (0.60–2.59)	1.29 (0.68–2.45)	0.97 (0.42–2.22)
1–4	36	15	13	1.66 (0.68–2.45)	0.99 (0.49–2.01)	1.67 (0.72–3.90)
5–9	41	15	25	1.37 (0.70–2.70)	1.50 (0.84–2.66)	0.92 (0.44–1.92)
≥10	174	68	79	1.27 (0.82–1.96)	0.89 (0.60–1.31)	1.42 (0.87–2.34)
Reproductive factors						
Parous						
Ever	361	125	164	1.0	1.0	
Never	101	34	48	1.06 (0.67–1.66)	1.21 (0.81–1.82)	0.87 (0.52–1.46)
Age at first birth (each additional year)				1.02 (0.98–1.06)	1.05 (1.02–1.09)	0.96 (0.92–1.01)
Children (number, among parous only)						
1	92	28	43	1.0	1.0	
2	161	59	82	1.18 (0.71–2.00)	1.05 (0.67–1.65)	1.03 (0.63–2.02)
≥3	108	38	39	1.13 (0.64–2.00)	0.73 (0.43–1.23)	1.55 (0.80–2.99)
Lactation (among parous women)						
Never	179	68	77	1.0	1.0	
Ever	177	57	86	0.92 (0.61–1.40)	1.29 (0.88–1.88)	0.72 (0.44–1.15)
Age at menarche (years)						
8–12	230	88	121	1.0	1.0	
≥13	232	71	91	0.80 (0.56–1.16)	0.73 (0.52–1.10)	1.10 (0.72–1.67)
Other factors						
Family history of breast cancer						
None	431	136	179	1.0	1.0	
First degree	31	23	33	2.27 (1.28–4.03)	2.44 (1.44–4.12)	0.93 (0.52–1.67)
Previous biopsy						
None	440	145	187	1.0	1.0	
≥1	22	14	25	1.89 (0.94–3.80)	2.52 (1.38–4.60)	0.75 (0.37–1.50)
Body size (body mass index)						
<23	171	64	87	1.0	1.0	
23–26	149	49	58	0.82 (0.53–1.29)	0.68 (0.45–1.02)	1.21 (0.73–2.01)
≥27	142	46	67	0.80 (0.51–1.26)	0.80 (0.54–1.19)	1.00 (0.61–1.66)
Physical activity (average of three time periods, relative units in quartiles)						
1	113	40	50	1.0	1.0	
2	119	41	55	1.00 (0.60–1.67)	1.09 (0.69–1.74)	0.92 (0.51–1.65)
3	115	36	54	0.92 (0.54–1.55)	1.13 (0.71–1.80)	0.81 (0.45–1.48)
4	115	42	53	1.08 (0.65–1.79)	1.12 (0.70–1.78)	0.97 (0.54–1.73)
Caloric intake (kilocalories, in quartiles)						
<1100	125	32	48	1.0	1.0	
1100–1450	113	41	59	1.42 (0.82–2.44)	1.53 (0.94–2.47)	0.93 (0.50–1.72)
1450–1830	112	32	51	1.12 (0.64–1.98)	1.34 (0.82–2.19)	0.84 (0.44–1.61)
≥1830	112	54	54	1.94 (1.15–3.28)	1.45 (0.89–2.37)	1.34 (0.73–2.46)
Education						
High school/Technical class	160	45	74	1.0	1.0	
Some college	116	42	55	1.32 (0.81–2.14)	1.06 (0.69–1.62)	1.24 (0.72–2.15)
College graduate	186	72	83	1.41 (0.92–2.17)	1.01 (0.69–1.49)	1.39 (0.85–2.27)
Race						
Whites	382	131	182	1.0	1.0	
Blacks	48	16	24	0.97 (0.53–1.77)	1.06 (0.63–1.78)	0.92 (0.47–1.80)
Asians and others	32	12	6	1.13 (0.56–2.26)	0.40 (0.17–0.99)	2.78 (1.02–7.61)

Table 2 Continued

	Controls (n = 462)	HER-2/*neu*+ cases (n = 159)	HER-2/*neu*− cases (n = 212)	Age-adjusted OR (95% CI)		Ratio of the ORs (95% CI)
				HER-2/*neu*+	HER-2/*neu*−	
Environmental factors						
Cigarette smoking						
Never	248	81	103	1.0	1.0	
Former	100	43	58	1.31 (0.84–2.02)	1.37 (0.92–2.05)	0.95 (0.58–1.55)
Current	113	35	51	0.95 (0.60–1.50)	1.11 (0.74–1.66)	0.86 (0.51–1.44)
Duration of smoking[b] (pack-years)						
<5	69	27	36	1.23 (0.74–2.06)	1.30 (0.81–2.07)	0.95 (0.53–1.70)
5–15	73	24	36	1.03 (0.60–1.72)	1.20 (0.76–1.91)	0.84 (0.47–1.53)
≥16	71	27	37	1.12 (0.67–1.87)	1.21 (0.76–1.92)	0.93 (0.52–1.65)
Age started smoking[b] (in years)						
8–16	66	15	21	0.71 (0.38–1.31)	0.78 (0.45–1.34)	0.91 (0.44–1.87)
16–17	55	21	33	1.18 (0.67–2.07)	1.48 (0.90–2.42)	0.80 (0.43–1.48)
≥18	92	42	55	1.37 (0.88–2.14)	1.41 (0.94–2.13)	0.97 (0.59–1.60)
Alcohol use (drinks/week)						
None	197	8	72	1.0	1.0	
<7	227	75	119	0.95 (0.65–1.40)	1.43 (1.0–2.04)	0.67 (0.43–1.03)
≥7	38	16	21	1.24 (0.65–2.36)	1.54 (0.84–2.80)	0.81 (0.39–1.67)
Electric blanket and mattress pad use						
Never	325	100	141	1.0	1.0	
Ever	137	59	71	1.38 (0.95–2.03)	1.17 (0.83–1.66)	1.18 (0.77–1.82)
Electric blanket and mattress pad use (in months)						
Never	325	100	141	1.0	1.0	
1–9	41	21	19	1.65 (0.93–2.92)	1.04 (0.58–1.86)	1.58 (0.81–3.10)
10–29	46	13	31	0.92 (0.48–1.77)	1.54 (0.94–2.54)	0.59 (0.30–1.19)
≥30	50	25	21	1.60 (0.94–2.73)	0.94 (0.54–1.63)	1.70 (0.90–3.21)

[a] Relative to never users.
[b] Relative to never smokers.

Table 3 Multivariate-adjusted[a] ORs and 95% CIs for HER-2/*neu*-positive (+) and HER-2/*neu*-negative (−) breast cancer among women <45 years of age in New Jersey, 1990–1992

	HER-2/*neu*+ OR (95% CI)	HER-2/*neu*− OR (95% CI)	Ratio of the ORs (95% CI)
Age at first use of OCs (in years)			
Never users	1.0	1.0	
<18	1.89 (0.97–3.85)	0.97 (0.47–2.00)	1.99 (0.87–4.54)
18–21	1.09 (0.68–1.77)	1.24 (0.82–1.87)	0.88 (0.52–1.50)
22+	1.46 (0.88–2.42)	0.84 (0.52–1.36)	1.75 (0.98–3.12)
Body mass index			
<23	1.0	1.0	
23–26	0.80 (0.50–1.26)	0.71 (0.46–1.08)	1.13 (0.67–1.90)
27+	0.76 (0.47–1.23)	0.80 (0.52–1.22)	0.96 (0.56–1.62)
Age at first birth (for each additional year)	1.02 (0.97–1.06)	1.06 (1.02–1.10)	0.96 (0.91–1.01)
Parous			
Ever	1.0	1.0	
Never	1.03 (0.63–1.68)	1.27 (0.82–1.96)	0.81 (0.47–1.40)
Age at menarche			
8–12	1.0	1.0	
13+	0.73 (0.49–1.07)	0.64 (0.45–0.91)	1.14 (0.74–1.77)
Family history			
None	1.0	1.0	
First degree	2.13 (1.16–3.91)	2.25 (1.29–3.91)	0.95 (0.52–1.75)
Prior breast biopsy			
No	1.0	1.0	
Yes	2.08 (0.98–4.42)	2.65 (1.36–5.17)	0.78 (0.37–1.65)
Caloric intake (kilocalories, in quartiles)			
<1100	1.0	1.0	
1100–1450	1.44 (0.82–2.51)	1.52 (0.92–2.51)	0.95 (0.50–1.78)
1450–1830	1.02 (0.56–1.85)	1.32 (0.79–2.22)	0.77 (0.39–1.51)
≥1830	2.04 (1.19–3.52)	1.57 (0.95–2.64)	1.29 (0.69–2.42)

[a] Adjusted for age and all other variables in the Table.

Table 4 Multivariate-adjusted[a] ORs and 95% CIs for HER2/*neu*-positive (+) and HER2/*neu*-negative (−) breast cancer in relation to patterns of OC use by estrogen receptor status

	Controls (n = 462)	HER2/*neu*+ (n = 62)	HER2/*neu*− (n = 109)	HER2/*neu*+ OR (95% CI)	HER2/*neu*− OR (95% CI)	Ratio of the ORs (95% CI)
		Among cases with ER+ tumors				
OC use						
Never	168	21	40	1.0	1.0	
Ever	294	41	69	0.99 (0.55–1.80)	0.93 (0.58–1.47)	1.07 (0.54–2.12)
OC duration (years)[b]						
<5	37	22	41	0.88 (0.44–1.74)	0.89 (0.53–1.50)	0.99 (0.45–2.16)
5–9	81	11	18	0.97 (0.42–2.25)	0.90 (0.47–1.73)	1.08 (0.41–2.82)
≥10	176	8	10	1.56 (0.59–4.13)	1.17 (0.52–2.67)	1.33 (0.43–4.13)
Age at first use of OC (in years)[b]						
<18	40	6	7	1.32 (0.44–3.99)	1.12 (0.44–2.84)	1.18 (0.32–4.35)
18–21	152	18	39	0.79 (0.39–1.62)	0.95 (0.56–1.62)	0.83 (0.37–1.86)
≥22	102	17	23	1.20 (0.58–2.51)	0.84 (0.46–1.53)	1.44 (0.61–3.39)
Number of years since first use[b]						
<15	86	10	13	0.91 (0.36–2.34)	0.78 (0.36–1.69)	1.17 (0.39–3.57)
15–19	113	15	20	0.77 (0.35–1.67)	0.67 (0.36–1.26)	1.14 (0.46–2.84)
≥20	95	16	36	1.39 (0.62–3.11)	1.36 (0.74–2.47)	1.03 (0.42–2.50)
Number of years since last use[b]						
<5	79	13	15	1.71 (0.74–3.95)	1.09 (0.52–2.26)	1.57 (0.58–4.28)
5–9	41	4	9	0.84 (0.26–2.74)	0.95 (0.40–2.23)	0.89 (0.24–3.34)
≥10	174	42	45	0.79 (0.39–1.57)	0.87 (0.51–1.46)	0.91 (0.42–1.99)
		Among cases with ER− tumors				
OC use						
Never	168	13	22	1.0	1.0	
Ever	294	51	34	2.58 (1.31–5.10)	0.92 (0.49–1.71)	**2.81 (1.18–6.67)**
OC duration (years)[b]						
<5	37	32	20	2.89 (1.40–5.97)	0.95 (0.47–1.92)	**3.04 (1.18–7.82)**
5–9	81	12	9	1.90 (0.78–4.58)	0.82 (0.34–1.99)	2.31 (0.72–7.46)
≥10	76	7	5	2.92 (1.03–8.29)	1.02 (0.31–3.29)	2.87 (0.66–12.45)
Age at first use of OC (in years)[b]						
<18	40	11	3	3.72 (1.44–9.63)	0.69 (0.19–2.53)	**5.37 (1.20–24.01)**
18–21	152	20	23	2.01 (0.93–4.34)	1.29 (0.65–2.55)	1.55 (0.59–4.08)
≥22	102	20	8	2.95 (1.34–6.49)	0.50 (0.19–1.32)	**5.92 (1.81–19.36)**
Number of years since first use[b]						
<15	86	17	12	2.96 (1.27–6.92)	1.21 (0.51–2.87)	2.44 (0.80–7.46)
15–19	113	18	10	2.15 (0.95–4.85)	0.64 (0.26–1.55)	**3.38 (1.08–10.56)**
≥20	95	16	12	2.76 (1.12–6.77)	0.98 (0.41–2.35)	2.81 (0.87–9.11)
Number of years since last use[b]						
<5	79	13	10	2.76 (1.13–6.73)	1.35 (0.54–3.38)	2.05 (0.62–6.77)
5–9	41	10	9	3.36 (1.31–8.63)	1.52 (0.58–3.98)	2.21 (0.64–7.61)
≥10	174	28	5	2.29 (1.07–4.89)	0.64 (0.30–1.37)	**3.58 (1.30–9.83)**

[a] Adjusted for age, body mass index, age at first birth, parity status, age at menarche, first-degree family history of breast cancer, prior breast biopsy, and caloric intake.
[b] Relative to never user.

been clinically demonstrated that gene protein overexpression assessed by immunohistochemistry, which has been shown to be associated with gene amplification, is related to worse prognosis and differential treatment responsiveness and is correlated with high tumor grade, large size, positive nodal status, ductal infiltration, histological type, and low values of estrogen and progesterone receptors (5, 17, 18). Whether HER-2/*neu* status can help to identify etiologically distinct subgroups of breast cancer cases has received only limited attention (8, 9, 19).

In the study reported here, the OR for breast cancer in relation to OC use before age 18 was elevated among women with HER-2/*neu*-positive tumors and decreased among women with HER-2/*neu*-negative tumors. The 2-fold heterogeneity in the ORs was statistically significant in age-adjusted models but not in multivariate-adjusted models. With further stratification by ER status, the ratio of the OR increased to 5-fold among women with tumors that were ER−, which reflects over a 3-fold increase in risk among women with HER-2/*neu*-positive tumors and a 31% decrease among women with HER-2/*neu*-negative tumors. There was little or no heterogeneity in relation to other risk factors, including age at first birth.

Interpretation of these results must be considered in light of the limitations and strengths of our study. The study sample was population based, which would decrease the likelihood of ascertainment bias. Also, there was little difference in the distribution of known and suspected risk factors between cases with and without archived tumor tissue available for our laboratory assays (11). In addition, the structured interview was developed to specifically assess OC use among young women and was administered by trained interviewers using a reproductive calendar to enhance recall (3).

Drawbacks to consider include the possibility that chance may account for some of the pattern of findings in our study. However, the variable for which our results are strongest is the one for which there is empirical support from previous research. Thus, our data confirm and expand upon an earlier observation of a large

increase in breast cancer risk in relation to OC use at an early age and HER-2/*neu* status that was reported previously by Olsson *et al.* (8) in 1991. However, we did not corroborate the earlier finding by Treurniet *et al.* (9) in which a 4-fold increase in risk in relation to age at first birth or breastfeeding was noted among women with HER-2/*neu*-positive tumors. A third study (19) found no association between HER-2/*neu* status in women with node-negative breast cancer and four risk factors examined, menstrual status, family history of breast cancer, age at first pregnancy, and number of pregnancies. Generalizability for all of these studies was hindered by a very select group of study subjects. In our study, our larger, population-based sample size permitted a more thorough and generalizable exploration of reproductive factors, as well as other risk factors for breast cancer, in relation to HER-2/*neu* status.

For a large epidemiological study, assessment of HER-2/*neu* protein overexpression by immunohistochemistry is a more cost-efficient method than assessing amplification or specific mutations. However, use of immunohistochemistry may have resulted in some misclassification of HER-2/*neu* status, although the correlation between amplification and overexpression is high (5, 17, 20). Olsson *et al.* (8) determined gene amplification and reported similar findings to those shown here. Also, in our population-based sample of young women <45 years of age, 43.9% of breast cancer cases showed evidence of HER-2/*neu* overexpression in the archived tumor tissue, which is within the 18–50% range reported by others (6, 9, 19, 21, 22).

In a recent large pooled analysis, the risk of breast cancer was found to be modestly elevated in relation to OC use (1), particularly long-term use, recent use, or use at an early age. However, there appears to be some heterogeneity in risk among certain subgroups, with the magnitude of risk higher among black women or among women with a family history of breast cancer (2, 3). Our study, however, had few nonwhite subjects to explore possible heterogeneity in the association between OC use and breast cancer risk stratified by race and with the cases categorized by HER-2/*neu* status.

Olsson (23) hypothesized that because both early age at first use of OCs and HER-2/*neu* amplification were associated with a shared tumor biology (larger tumor size, advanced tumor stage, absence of steroid receptors, a higher rate of proliferation, and high tumor grade), it is possible that the exposure and gene amplification were related. In addition, the strong association between patterns of OC use and HER-2/*neu* positivity among women with ER-negative tumors noted in our study may be biologically plausible. Because antiestrogens lower HER-2/*neu* levels in ER-tumors (24), it is plausible that estrogens stimulate HER-2/neu in these tumors. Thus, variation in the distribution of ER status in populations could result in heterogeneous results when examining the relation between OCs and HER-2/*neu*+ breast cancer. Thus, failure to consider HER-2/*neu* and ER status could mask any strong, underlying association between OCs and breast cancer risk.

In summary, this study of young women confirms the association first noted by Olsson *et al.* (8) of a heterogeneity of effect for breast cancer in relation to OCs when cases are stratified by HER-2/*neu* status. This study is the first to report a significant 3-fold increase in risk associated with oral contraceptive use among young women with tumors that are HER-2/*neu* positive and ER negative. Further corroboration by others is needed to examine these provocative associations among younger and older women with breast cancer.

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