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

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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

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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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