Environmental Factors in Relation to Breast Cancer Characterized by p53 Protein Expression[1]

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Abstract

Findings from studies of cigarette smoking and low-dose ionizing radiation exposure and breast cancer are unclear. Laboratory studies indicate that both exposures can cause DNA damage, potentially increasing cancer risk if such mutations occur in growth control genes, such as *p53*. We examined the potential etiologic heterogeneity of breast cancer by evaluating whether associations between cigarette smoking and low-dose ionizing radiation and breast cancer differed by p53 protein expression status. Data were obtained from the Carolina Breast Cancer Study, a population-based, case-control study conducted among African-American and white women ages 20–74 years. Questionnaire data were available from 861 women with incident, primary invasive breast cancer and 790 community-based controls. p53 immunostaining was performed on tissue from 683 women with breast cancer; 46% were classified as p53+. Two separate unconditional logistic regression models were used to calculate odds ratios (ORs) for p53+ and p53− breast cancer, as compared with controls, in relation to smoking and low-dose ionizing radiation exposure. Smoking was not differentially associated with p53+ or p53− breast cancer, even when duration, dose, and passive smoking status were considered. Exposure to individual sources of radiation did not differ for p53+ and p53− breast cancers. However, ORs for combined exposure to chest X-rays and occupational radiation were higher for p53+ [OR, 2.2; 95% confidence interval (CI), 1.0–5.3] than p53− breast cancer (OR, 1.2; 95% CI, 0.5–3.4). Combined exposure to radiation from other medical sources as well as occupational exposure was also higher for p53+ (OR, 3.7; 95% CI, 0.8–16.8) than for p53− breast cancer (OR, 1.7; 95% CI, 0.3–10.5). Although preliminary, our results suggest that exposure to multiple sources of low-dose ionizing radiation may contribute to the development of p53+ breast cancer.

Introduction

Findings from epidemiological studies of cigarette smoking, low-dose ionizing radiation exposure, and breast cancer risk are inconsistent. Most studies of smoking and breast cancer report null or weak positive associations limited to specific subgroups of women (1–12), whereas others report weak inverse associations (13–15). Although high-dose ionizing radiation exposure is considered an established cause of breast cancer based on studies of female survivors of the atomic bomb explosions in Japan (16), the effect of low-dose ionizing radiation exposure from more common sources, such as chest X-rays, is not clear.

Laboratory studies indicate that both tobacco smoke carcinogens and ionizing radiation cause DNA damage, potentially increasing cancer risk if such mutations occur in critical regions of growth control genes, such as the tumor suppressor gene *p53*. p53 regulates cellular proliferation and apoptosis and may contribute to cancer when inactivated or altered by mutations or other mechanisms (17). Mutational spectra analyses in lung tumors reveal associations between benzo(*a*)pyrene in tobacco smoke and signature *p53* mutations, in particular G to T transversions at codons 157, 158, 248, and 273 (18–20). Radiation exposure is associated with deletion and missense mutations (G to T transversions) in *p53* in studies of lung tumors (21, 22) and with impaired p53-mediated response to DNA damage in ataxia telangiectasia mutation carriers in breast cancer (23, 24).

The prevalence of *p53* mutations in breast cancer is ~30% (18, 25). The mutational spectra of breast cancer is similar to that of lung cancer in that they both exhibit high proportions of missense mutations, but the distribution and position of transversion and transition mutations differ (26). Conway *et al.* (27) described recently the prevalence and spectrum of *p53* mutations among a subset of cases from the CBCS[3] ($n = 456$). A higher proportion of G to T transversion mutations were found in the breast tumors of current smokers compared with those of former and never smokers. Accumulation of the p53 protein is detected in ~45% of breast cancers, suggesting that p53 function can be altered by mechanisms other than mutation (28, 29). It is also possible for *p53* mutations to occur outside of the sequenced region, which IHC protein staining will detect if the mutation results in stabilization (18). IHC detection of the p53 protein is more practical for large-scale epidemiological studies

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[3] The abbreviations used are: CBCS, Carolina Breast Cancer Study; OR, odds ratio; CI, confidence interval; IHC, immunohistochemistry; ER, estrogen receptor.

and may be useful in identifying factors that operate through a p53 pathway to influence breast cancer risk.

Subdividing breast cancers by p53 status may elucidate associations among smoking, low-dose ionizing radiation, and breast cancer risk (30). Only two population-based, case-control studies of breast cancer have examined p53 protein expression in breast tumors with respect to etiology, and both focused on younger women (31, 32). The present analysis evaluated whether associations between environmental exposures such as cigarette smoking and low-dose ionizing radiation exposure differed for p53+ and p53− breast cancer in relation to controls in the CBCS, a large population-based study ($n = 683$) that included a diverse population 20–74 years of age.

Materials and Methods

Data were obtained from the CBCS, a population-based, case-control study of breast cancer conducted among African-American and white women ages 20–74 years in North Carolina (33). Incident cases of invasive breast cancer were identified through a rapid case ascertainment system in cooperation with the North Carolina Central Cancer Registry (34). Eligible cases included women who were diagnosed with their first case of invasive breast cancer between May 1, 1993 and May 30, 1996. Controls were identified from North Carolina Division of Motor Vehicle and United States Health Care Financing Administration lists. Randomized recruitment was used to frequency-match controls to cases by race (African-American or white) and age (in 5-year age categories), and to oversample women <50 years of age and African-American women (35).

Data were collected by trained nurse interviewers who administered a questionnaire at the participant's home, took body size measurements, and drew a 30-ml blood sample. The questionnaire included assessment of each participant's menstrual, reproductive, and contraceptive histories, first-degree family history of breast cancer, occupational history, exposure to occupational and medical ionizing radiation, and other lifestyle factors. Interviews were completed on 77% ($n = 861$) of eligible and locatable cases and 68% ($n = 790$) of eligible and locatable controls (36). In the parent study, risk factors associated with slight increased risk of breast cancer included younger age at menarche, oral contraceptive use, history of breast or ovarian cancer in first-degree relatives, higher education level, former smoking status, and long-term smoking and low-dose ionizing radiation exposures. Ever having a full-term pregnancy, lactation among parous women, and higher body mass index were inversely associated with breast cancer overall; minor differences were observed when participants were stratified by race and/or menopausal status (7, 37–40).

With informed patient consent, 738 tumor blocks containing invasive breast cancer (86%) were received from pathology departments from participating hospitals and processed by the CBCS. Hospital slides were retrieved for an additional 69 cases (8%), leaving 54 cases for whom no clinical specimen was obtained (6%). Blocks were sectioned according to the defined study protocol (41), and case slides were assayed within 4 weeks of being sectioned. A total of 683 case slides (79%) were assayed for p53 protein expression using an IHC technique that uses an antibody with high sensitivity in paraffin-embedded tissues (DO7; DAKO, Glostrup, Denmark). Positive and negative control slides were included with each batch. Formalin-fixed, paraffin-embedded tissue sections were placed on coated slides and baked at 60°C for 1 h, deparaffinized in xylene, hydrated in descending alcohols, placed in an antigen retrieval Citra buffer (pH 6.0), and heated in a microwave oven to subboiling for 10 min. Normal horse serum blocking solution was subsequently applied to the cooled slides to block the sites to which the secondary antibody was expected to bind. The primary p53 mouse monoclonal antibody clone DO7 was applied to all of the slides and incubated in a humidity chamber for 1 h. Negative antibody control slides were incubated with nonspecific IgE under identical conditions. A secondary biotinylated antibody (horse, antimouse) application followed. An avidin-biotin complex application sought out the biotin from the secondary antibody and prepared the slides for the color deposit. The chromagen 3,3′-diaminobenzidine was used, and finally the slides were counterstained with hematoxylin.

Slides were read by the study pathologist (J. G.) who recorded detailed information on the intensity, localization and distribution of the protein stain. Cases were considered p53+ if dark nuclear protein staining was present in 10% or more of the invasive tumor cells; cases with <10% cells of dark, nuclear staining were considered p53−.

Race was classified according to self-report. Fewer than 2% of women described themselves as races other than African-American or white and were classified as white. Women were considered postmenopausal if they had undergone natural menopause, bilateral oophorectomy, or if they were 50 years and older and had ceased menstruating or were taking hormone replacement therapy. Participants were classified as smokers if they reporting smoking >100 cigarettes in their lifetime. Living with a smoker was regarded as passive smoking exposure. Exposure to low-dose ionizing radiation was captured by several variables including: chest X-ray before age 20 (yes/no), medical treatment-related radiation (yes/no for exposure anywhere in the body to monitor or treat a condition other than breast cancer such as scoliosis, chest fluoroscopy for tuberculosis, or kidney stones), and occupational exposure to ionizing radiation (based on participant's report of two longest held jobs since age 18). The International Commission of Radiological Protection classification was used to classify jobs with potential exposure and included registered nurses, licensed practical nurses, medical doctors, and radiological technicians (40, 42).

χ^2 statistics were used to test differences in proportions, using SAS 6.0 (43). Unconditional logistic regression was used to calculate ORs and 95% CIs for p53+ breast cancer cases and p53− breast cancer cases as compared with controls in relation to cigarette smoking and low-dose ionizing radiation exposure. PROC GENMOD was used to adjust for age (as an 11-level ordinal variable reflecting 5-year age categories), race (African-American or white), and to incorporate offset terms derived from sampling fractions used to identify eligible participants. Multivariable logistic regression models were used to adjust for potential confounding factors, although ORs were not altered significantly after adjustment for additional covariates. Therefore, ORs shown are adjusted only for design variables (age and race) and sampling fractions.

Tumor stage and hormone receptor status information was abstracted from the medical records of the cases. Tumor stage was evaluated as a multilevel variable based on the American Joint Classification of Cancer categories (44). ER status was abstracted from medical records for the majority of the cases. For 11% of the cases, ER status was determined in our IHC laboratory using a positive cutpoint of 5% (45). Case-case analyses (p53+ cases *versus* p53− cases) provided information about the magnitude of the degree of heterogeneity between the two disease subgroups (p53+ and p53− breast cancers) and permitted adjustment for tumor characteristics in the models (46).

Table 1 Characteristics of cases on whom p53 immunostaining was performed compared with cases on whom staining information was not available

	Cases without p53 information		Cases with p53 information		*P*
	(*n* = 178)	%	(*n* = 683)	%	
Age					
<50	101	56.7	404	59.2	0.56
≥50	77	43.3	279	40.8	
Race					
White	120	67.4	406	59.4	0.05
African-American	58	32.6	277	40.6	
Stage at diagnosis					
I	83	54.6	250	39.3	0.002
IIA	52	34.2	311	48.9	
IIB–IV	17	11.2	75	11.8	
Hormone receptor status					
ER+	84	61.3	379	58.0	0.48
ER−	53	38.7	274	42.0	
Age at menarche (yr)					
<13	86	48.6	352	51.5	0.48
≥13	91	51.4	331	48.5	
Ever had full-term pregnancy					
Yes	148	83.2	580	84.9	0.56
No	30	16.8	103	15.1	
Oral contraceptive use status					
Current use	12	6.8	44	6.5	0.20
Former use	112	63.3	384	56.4	
Never used	53	29.9	253	37.1	
Menopausal status					
Premenopausal	89	50.0	353	51.7	0.69
Postmenopausal	89	50.0	330	48.3	
Family history of breast cancer					
Yes	35	20.4	91	13.7	0.03
No	137	79.6	574	86.3	
Education level attained					
≤High school	67	37.7	329	48.2	0.01
Tech/Business school	46	25.8	176	25.8	
≥College	65	36.5	178	26.0	
Ever smoker					
Yes	87	48.9	330	48.3	0.89
No	91	51.1	353	51.7	
Active smoking status					
Current smoker	24	13.5	128	18.7	0.15
Former smoker	63	35.4	202	29.6	
Never smoked	91	51.1	353	51.7	
Usual amount smoked (packs/day)					
≤½ pack	24	13.5	117	17.2	0.35
½ to 1 pack	31	17.4	119	17.5	
>1 pack	32	18.0	91	13.4	
Never smoked	91	51.1	353	51.9	
Duration of smoking (yr)					
<10	24	13.5	95	13.9	0.27
11–20	27	15.2	70	10.3	
>20	36	20.2	164	24.1	
Never smoker	91	51.1	353	51.7	
Exposure to passive smoking					
Yes	156	88.1	595	87.2	0.75
No	21	11.9	87	12.8	
Combination smoking exposure					
No passive, no active	14	7.9	64	9.4	0.91
Passive, no active	77	43.5	288	42.2	
No passive, active	7	4.0	23	3.4	
Passive and active	79	44.6	307	45.0	
Chest X-ray before age of 20					0.63
Yes	45	26.0	492	27.9	
No	128	74.0	190	72.1	
Other medical sources of low-dose ionizing radiation					
Yes	17	90.4	62	90.9	0.85
No	161	9.6	621	91.0	
Occupational radiation exposure					
Yes	13	7.3	46	6.7	0.79
No	165	92.7	637	93.3	

Results

Of the 861 cases who were interviewed for the CBCS, 683 tumor specimens were obtained and assayed for p53 (79%). As shown in Table 1, cases missing p53 information were diagnosed with a lower stage of breast cancer and had a higher education level than cases with p53 information ($P < 0.05$) but were similar with respect to smoking and radiation exposure variables.

The prevalence of p53 protein staining among subgroups of CBCS cases are shown in Table 2. Overall, 46.4% of cases were classified as p53+ in the study sample. The p53+ cases, in contrast to p53− cases, were more likely to be younger ($P < 0.001$), premenopausal ($P < 0.0002$), have a first-degree family history of breast cancer ($P = 0.14$), and be diagnosed with later stage disease ($P = 0.06$). An inverse pattern was noted between p53 status and ER status; p53+ cases were more likely to be ER− ($P = 0.03$). When p53 staining distributions were compared for African-American and white cases by menopausal status, similar patterns were observed; ~50% of premenopausal African-American and white cases had p53+ breast cancer, whereas ~40% of postmenopausal cases were p53+.

p53+ and p53− breast cancer cases were compared with controls in relation to cigarette smoking and low-dose ionizing radiation exposure, as shown in Table 3. Analyses were initially performed separately for premenopausal and postmenopausal women and also for African-American and white women, because breast cancer etiology might differ for these subgroups. Patterns were similar by menopausal status and race; therefore, findings presented here are for all groups combined.

The association between cigarette smoking and breast cancer did not differ by p53 status in this study. No association was observed whenever smokers were compared with never smokers for p53+ and p53− breast cancer relative to controls. Former smoking, as compared with never smoking, was associated with slight increases in the ORs for both p53+ and p53− case groups. A significant but small positive association was observed for p53− breast cancer and long-term smoking. The OR for p53− breast cancer comparing women who had smoked ≥20 years to nonsmokers was 1.5 (95% CI, 1.1–2.1), whereas the corresponding OR for p53+ breast cancer was somewhat similar, although not statistically significant (1.3; 95% CI, 0.9–1.8). No dose-response patterns were noted across levels of smoking duration (total years smoked) or dose (packs/day). Accounting for passive smoking exposure did not reveal significant breast cancer heterogeneity by p53 status; ORs were similar for both case groups.

ORs were slightly higher for p53+ breast cancer than for p53− breast cancer with respect to exposure to certain sources of low-dose ionizing radiation. As shown in Table 3, having a chest X-ray before the age of 20 was slightly associated with p53+ breast cancer but not p53− breast cancer. No association was observed for medical treatment-related ionizing radiation exposure and p53+ or p53− breast cancer, whereas occupational exposure to ionizing radiation was associated with ORs

Table 2 Selected demographic and tumor characteristics by p53 expression status among breast cancer cases in the CBCS, 1993–1996

	p53+ cases n = 317 (46.4%)	p53− cases n = 366 (53.6%)	P
	n (%)	n (%)	
Age			
<50 yr	208 (65.6)	196 (53.5)	0.001
≥50 yr	109 (34.4)	170 (46.5)	
Race			
White	184 (58.0)	222 (60.7)	0.49
African-American	133 (42.0)	144 (39.3)	
Menopausal status			
Premenopausal	188 (59.3)	165 (45.1)	0.0002
Postmenopausal	129 (40.7)	201 (54.9)	
Family history of breast cancer[a]			
Yes	49 (15.8)	42 (11.9)	0.14
No	262 (84.2)	312 (88.1)	
Stage at diagnosis			
I	103 (35.0)	147 (43.0)	0.06
IIA	92 (31.3)	95 (27.8)	
IIB–IV	99 (33.7)	100 (29.2)	
Estrogen receptor status			
ER+	162 (53.5)	217 (62.0)	0.03
ER−	141 (46.5)	133 (38.0)	

[a] Family history of breast cancer in a first-degree relative.

of 1.9 for both p53+ and p53− breast cancer. A variable representing whether participants had none, one, or two or more types of low-dose radiation exposure was constructed and examined in relation to p53 breast cancer subtypes. ORs were slightly higher for p53+ breast cancer than p53− breast cancer with respect to the number of sources of low-dose ionizing radiation exposure [p53+ ORs, 1.2 (95% CI, 0.9–1.7) for one source and 1.5 (95% CI, 0.8–2.6) for two or more sources; p53− ORs, 1.0 (95% CI, 0.8–1.3) and 1.3 (95% CI, 0.7–2.2), respectively]. Because this variable did not distinguish between sources of exposure, further analyses were conducted to determine the contributions of medical treatment-related, chest X-ray, and occupational radiation exposures. Exposure to both chest X-ray and occupational radiation was more strongly associated with p53+ than p53− breast cancer relative to no exposure (p53+ OR, 2.2; 95% CI, 1.0–5.3; and p53− OR, 1.3; 95% CI, 0.5–3.4). The combination of medical treatment-related ionizing radiation and occupational exposure resulted in ORs for p53+ breast cancer that were higher in magnitude (OR, 3.7; 95% CI, 0.8–16.8) than for p53− breast cancer (OR, 1.7; 95% CI, 0.3–10.5) also. This pattern did not persist when medical treatment-related radiation and chest X-rays were evaluated together. Adjustment for stage and ER status did not substantially alter any of the risk estimates for smoking or low-dose radiation exposure in case-only analyses.

Discussion

The results from this population-based study of women 20–74 years of age suggest that cigarette smoking and low-dose ionizing radiation exposure are not differentially associated with p53+ and p53− breast cancer. Although associations for p53+ breast cancer were slightly stronger than those for p53− breast cancer when certain sources of low-dose ionizing radiation exposure were combined, these results are preliminary and should be confirmed in other studies. The inverse association noted for p53 status and ER status is in accordance with numerous studies in the pathologic literature (47–49).

The relationship between low-dose ionizing radiation exposure and p53+ and p53− breast cancers has not been reported previously. High-dose radiation exposure, such as that endured by Hiroshima survivors, is associated with increased risk of breast cancer (16). Evidence also exists to indicate a higher breast cancer incidence and mortality among women who underwent diagnostic or therapeutic irradiation for a variety of conditions including benign breast disease, enlarged thymus, scoliosis, and postpartum mastitis, especially during adolescence (50–54). It is possible that the elevated ORs for p53+ breast cancer associated with low-dose ionizing radiation exposure is identifying carriers of ataxia telangiectasia mutations who are extremely radiosensitive or individuals who have inefficient DNA repair capabilities.

The ionizing radiation variables that were evaluated in this population-based study included those deriving from medical procedures (such as chest X-rays), medical treatment (such as irradiation for an enlarged thymus), and those obtained while on the job. In addition, we evaluated whether multiple sources of radiation exposure influenced development of p53+ and p53− breast cancer. Admittedly, sample sizes diminished quickly, and confidence intervals were wide and overlapping; results therefore are regarded as preliminary. Nevertheless, it is possible that low-dose ionizing radiation influences breast cancer risk by adversely affecting the *p53* gene, and OR patterns are generally supportive of this idea.

Because the breast is hypothesized to be especially susceptible to DNA damage during puberty when cells are rapidly dividing (16, 55), we examined whether exposure to chest X-rays before the age of 20 increased risk for p53+ or p53− breast cancer. The proportion of cases reporting a chest X-ray before age 20 was higher among p53+ cases than p53− cases and controls, resulting in a slight positive association. However, the reference group was heterogeneous, consisting of those never exposed to a chest X-ray and those who reported having a chest X-ray sometime after age 20. If radiation exposure exerts a deleterious effect on the *p53* gene, it is possible that the OR for p53+ breast cancer is underestimated because the reference group contains women with the exposure.

Occupational exposure to radiation was associated with a doubling of risk for both p53+ and p53− breast cancer. When occupational exposure was combined with medical treatment-related radiation exposure or chest X-ray exposure, higher ORs were observed for p53+ breast cancer. This pattern was not seen when chest X-rays and medical treatment radiation were combined. The basis for this apparent cumulative effect is not clear, and because of the relatively small numbers in key strata, it is not possible to analyze this interaction further. Moreover, for most comparisons, confidence intervals overlap, and hence the radiation from medical sources may not be increasing risk of p53+ breast cancer beyond that experienced from occupational exposure.

Two previous studies examined the relationship between smoking and breast cancer by p53 expression status. Our findings differ from Gammon *et al.* (32), who noted a positive association between current smoking and p53+ breast cancer in their study of breast cancer among women <45 years (p53+ OR, 1.29; 95% CI, 0.79–2.11; p53− OR, 0.66; 95% CI, 0.41–1.06; ratio of the ORs, 1.96; 95% CI, 1.10–3.52). van der Kooy *et al.* (31) also reported an elevated OR for p53+ breast cancer for current smokers (p53+ OR, 1.4; 95% CI, 0.9–2.2; p53− OR, 0.9; 95% CI, 0.6–1.4) in their population-based study of younger women. Our results also differ from the conclusions of Conway *et al.* (27), who evaluated the prevalence and mutational spectrum of *p53* mutations in relation to smoking expo-

Table 3 Age- and race-adjusted ORs and 95% CIs for p53+ and p53− breast cancer in relation to cigarette smoking and low-dose ionizing radiation exposure among CBCS participants, 1993–1996

	p53+ cases $n = 317$	p53− cases $n = 366$	Controls $n = 790$	p53+ *versus* controls OR (95% CI)[a]	p53− *versus* controls OR (95% CI)[a]	p53+ cases *versus* p53− cases OR (95% CI)[a]
Ever smoker						
Never smoked	167 (52.7)	186 (50.8)	423 (53.5)	1.0	1.0	1.0
Ever smoked	150 (47.3)	180 (49.2)	367 (46.5)	1.1 (0.8–1.4)	1.1 (0.9–1.5)	0.9 (0.7–1.3)
Active smoking status						
Never smoked	167 (52.7)	186 (50.8)	423 (53.5)	1.0	1.0	1.0
Current smoker	56 (17.7)	72 (19.7)	165 (20.9)	0.9 (0.6–1.2)	1.0 (0.7–1.4)	0.8 (0.6–1.3)
Former smoker	94 (29.6)	108 (29.5)	202 (25.6)	1.2 (0.9–1.7)	1.2 (0.9–1.7)	1.0 (0.7–1.4)
Usual amount smoked (packs/day)						
Never smoked	167 (53.0)	186 (51.0)	423 (53.6)	1.0	1.0	1.0
<½ pack	57 (18.1)	60 (16.4)	131 (16.6)	1.1 (0.8–1.6)	1.0 (0.7–1.5)	1.1 (0.7–1.6)
½ to 1 pack	59 (18.7)	60 (16.4)	119 (15.1)	1.3 (0.9–1.9)	1.2 (0.9–1.8)	1.1 (0.7–1.6)
>1 pack	32 (10.2)	59 (16.2)	116 (14.7)	0.7 (0.5–1.1)	1.1 (0.8–1.7)	0.6 (0.4–1.0)
Duration of smoking (yr)						
Never smoked	167 (52.8)	186 (50.8)	423 (53.6)	1.0	1.0	1.0
<10	47 (14.9)	48 (13.1)	105 (13.3)	1.0 (0.7–1.5)	0.9 (0.6–1.6)	1.0 (0.6–1.6)
11–20	34 (10.8)	36 (9.9)	100 (12.7)	0.8 (0.5–1.3)	0.8 (0.5–1.4)	1.0 (0.6–1.7)
>20	68 (21.5)	96 (26.2)	161 (20.4)	1.3 (0.9–1.8)	1.5 (1.1–2.1)	0.9 (0.6–1.3)
Exposure to passive smoking						
No	46 (14.5)	41 (11.2)	91 (11.5)	1.0	1.0	1.0
Yes	271 (85.5)	324 (88.8)	699 (88.5)	0.9 (0.6–1.3)	1.1 (0.8–1.7)	0.8 (0.5–1.3)
Combination smoking exposure						
No passive or active smoking	32 (10.1)	32 (8.8)	55 (7.0)	1.0	1.0	1.0
Passive smoking, no active	135 (42.6)	153 (41.9)	368 (46.5)	0.8 (0.5–1.2)	0.8 (0.5–1.3)	1.0 (0.6–1.7)
Active smoking	150 (47.3)	180 (49.3)	367 (46.5)	0.9 (0.5–1.4)	0.9 (0.6–1.5)	1.0 (0.6–1.7)
Chest X-ray before age 20						
No X-ray <20 yr	223 (70.6)	269 (73.5)	572 (73.3)	1.0	1.0	1.0
Yes, <20 yr	93 (29.4)	97 (26.5)	208 (26.7)	1.2 (0.9–1.6)	1.0 (0.8–1.4)	1.1 (0.8–1.6)
Other medical low-dose ionizing radiation exposure						
No	287 (90.5)	334 (91.3)	708 (89.6)	1.0	1.0	1.0
Yes	30 (9.5)	32 (8.7)	82 (10.4)	1.1 (0.7–1.7)	0.9 (0.6–1.3)	1.3 (0.7–2.2)
Occupational radiation exposure						
No	295 (93.1)	342 (93.4)	761 (96.3)	1.0	1.0	1.0
Yes	22 (6.9)	24 (6.6)	29 (3.7)	1.9 (1.1–3.4)	1.9 (1.1–3.3)	1.0 (0.6–1.9)
No. of sources of radiation exposure[b]						
None	191 (60.4)	234 (64.0)	500 (64.1)	1.0	1.0	1.0
One source	107 (33.9)	111 (30.3)	242 (31.0)	1.2 (0.9–1.7)	1.0 (0.8–1.3)	1.2 (0.9–1.7)
Two or more sources	18 (5.7)	21 (5.7)	38 (4.9)	1.5 (0.8–2.6)	1.3 (0.7–2.2)	1.1 (0.5–2.1)
Combined chest X-ray and occupational radiation exposure						
No	211 (66.7)	252 (68.9)	555 (71.2)	1.0	1.0	1.0
Yes, either	95 (30.1)	107 (29.2)	213 (27.3)	1.2 (0.9–1.7)	1.2 (0.9–1.5)	1.0 (0.7–1.5)
Yes, both	10 (3.2)	7 (1.9)	12 (1.5)	2.2 (1.0–5.3)	1.3 (0.5–3.4)	1.6 (0.8–4.2)
Combined other medical radiation and occupational radiation exposure						
No	269 (84.8)	312 (85.2)	682 (86.3)	1.0	1.0	1.0
Yes, either	44 (13.9)	52 (14.2)	105 (13.3)	1.2 (0.8–1.8)	1.1 (0.8–1.6)	1.1 (0.7–1.7)
Yes, both	4 (1.3)	2 (0.6)	3 (0.4)	3.7 (0.8–16.8)	1.7 (0.3–10.5)	2.4 (0.4–13.0)
Combined chest X-ray and other medical radiation exposure						
No	201 (63.6)	249 (68.0)	515 (66.0)	1.0	1.0	1.0
Yes, either	107 (33.9)	105 (28.7)	240 (30.8)	1.2 (0.9–1.7)	0.9 (0.7–1.2)	1.3 (0.9–1.8)
Yes, both	8 (2.5)	12 (3.3)	25 (3.2)	1.0 (0.5–2.3)	1.1 (0.5–2.2)	0.9 (0.4–2.2)

[a] Adjusted for age, race, and sampling fractions.
[b] "Source" includes chest X-ray before age 20, other medical radiation, and occupational radiation exposure.

sure in a subset of cases used in this analysis. They reported a higher prevalence of G→T transversion mutations in *p53* in the breast tumors of current smokers (5.9%) compared with never smokers (0.9%), with little difference for other types of mutations.

It is not clear why our results differ from the previous studies. Despite similar p53+ prevalences and even when analyses were restricted to younger women, no associations between smoking and p53+ breast cancer were observed in our study. It is possible that genetic differences in metabolism and detoxification of tobacco smoke carcinogens between study populations contribute to the disparate findings. However, as noted by Gammon *et al.*, it is also possible their results were attributable to chance because no dose-response relationships were observed. The results of van der Kooy *et al.* (32) are even less convincing of a difference between p53+ and p53− breast cancer (31). A potential explanation for the discordance between our findings and those of Conway *et al.* (27) is the low prevalence of the transversion mutations and the lack of specificity of the IHC technique. Only 5.9% (5 of 85) of the cases who were current smokers in Conway's study revealed G to T transversion mutations in *p53*, whereas in this study, 17.7%

(56/317) of the cases who were current smokers were classified as p53+ using IHC. Thus, the cases whose tumors stained positive for p53 as a result of other molecular mechanisms than mutation may be obscuring the association noted by Conway *et al.* (27).

Our findings should be interpreted in light of the study's limitations. Although size of the CBCS was nearly twice as large as either of the other studies, sample sizes were diminished after stratification of cases by p53 status and across multiple levels of exposure. This resulted in wide confidence limits for some variables. Additionally, p53 data were not available for all of the cases who contributed questionnaire information. However, it is unlikely that nonresponse to the study was related to p53 status; cases missing p53 information differed little from those with information.

Limitations resulting from IHC classification of p53 status must be acknowledged. Only one tumor slide was assayed per case, which may not adequately capture tumor tissue heterogeneity (56). Additionally, tumor specimens were retrieved from 26 participating hospitals, where potential variation in quality of fixation could have affected p53 staining results, probably lowering the proportion of p53+ tumors. However, the proportion of p53+ cases was similar to that of previous studies. Most importantly, IHC will not detect *p53* mutations that are so severe that no protein is made (18). An example of such a mutation is a deletion. Because ~7% of the CBCS cases had tumors containing deletion mutations in *p53*, the potential for misclassification by IHC is high in this group (27). Ionizing radiation exposure is associated with point mutations as well as *p53* deletions, which might result in misclassification among exposed cases. Cases with deletions were likely classified as p53− (because no protein is made), despite having an abnormality in the gene. This type of misclassification error may have attenuated the ORs for p53+ breast cancer and elevated associations for p53− breast cancer.

Strengths of this study include its population-based design with representation of younger and older women and the comprehensive questionnaire that included low-dose ionizing radiation exposure assessment. The tumor block acquisition rates were high, and immunostaining of p53 status enabled a larger sample size of cases to be evaluated with respect to several exposures.

In conclusion, the results from this study provide little evidence for breast cancer heterogeneity as classified by p53 expression status in relation to environmental exposures. Our findings of increased risk of p53+ breast cancer among women exposed to multiple sources of low-dose ionizing radiation exposure should be evaluated further. To elucidate specific mechanisms behind exposures believed to adversely affect the *p53* gene, analyses involving genetic sequencing could be performed.

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