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ARTICLE

Radiation Exposure, the *ATM* Gene, and Contralateral Breast Cancer in the Women's Environmental Cancer and Radiation Epidemiology Study

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Background Ionizing radiation is a known mutagen and an established breast carcinogen. The *ATM* gene is a key regulator of cellular responses to the DNA damage induced by ionizing radiation. We investigated whether genetic variants in *ATM* play a clinically significant role in radiation-induced contralateral breast cancer in women.

Methods The Women's Environmental, Cancer, and Radiation Epidemiology Study is an international population-based case–control study nested within a cohort of 52 536 survivors of unilateral breast cancer diagnosed between 1985 and 2000. The 708 case subjects were women with contralateral breast cancer, and the 1397 control subjects were women with unilateral breast cancer matched to the case subjects on age, follow-up time, registry reporting region, and race and/or ethnicity. All women were interviewed and underwent full mutation screening of the entire *ATM* gene. Complete medical treatment history information was collected, and for all women who received radiotherapy, the radiation dose to the contralateral breast was reconstructed using radiotherapy records and radiation measurements. Rate ratios (RRs) and corresponding 95% confidence intervals (CIs) were estimated by using multivariable conditional logistic regression. All *P* values are two-sided.

Results Among women who carried a rare *ATM* missense variant (ie, one carried by <1% of the study participants) that was predicted to be deleterious, those who were exposed to radiation (mean radiation exposure = 1.2 Gy, SD = 0.7) had a statistically significantly higher risk of contralateral breast cancer compared with unexposed women who carried the wild-type genotype (0.01–0.99 Gy: RR = 2.8, 95% CI = 1.2 to 6.5; ≥1.0 Gy: RR = 3.3, 95% CI = 1.4 to 8.0) or compared with unexposed women who carried the same predicted deleterious missense variant (0.01–0.99 Gy: RR = 5.3, 95% CI = 1.6 to 17.3; ≥1.0 Gy: RR = 5.8, 95% CI = 1.8 to 19.0; P_{trend} = .044).

Conclusions Women who carry rare deleterious *ATM* missense variants and who are treated with radiation may have an elevated risk of developing contralateral breast cancer. However, the rarity of these deleterious missense variants in human populations implies that *ATM* mutations could account for only a small portion of second primary breast cancers.

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Of the nearly 185 000 US women who were expected to develop breast cancer in 2009 and the more than 2.4 million breast cancer survivors who currently reside in the United States, 5%–10% will develop a subsequent primary cancer in the contralateral breast (www.cancer.org). Epidemiological studies have identified a number of factors that are associated with an increased risk of developing contralateral breast cancer, including family history of breast cancer, early age at diagnosis, hormonal factors, reproductive history, body mass index, and characteristics of the first primary tumor (eg, lobular histology, stage, and estrogen receptor status) (1–10). The risk of developing contralateral breast cancer has also been associated with mutations in specific genes, including *BRCA1*, *BRCA2*, and *CHEK2* (11–17). Importantly, the treatment a woman receives for her first primary breast cancer can also influence her risk of developing a second breast cancer: Chemotherapy is associated with a 40% reduction in the risk of developing contralateral breast cancer (1,18,19), whereas the radiation received to the contralateral breast during radiotherapy is associated with an increased risk of contralateral breast cancer (9,20,21). For example, we previously reported a threefold increase

CONTEXT AND CAVEATS

Prior knowledge

The ataxia telangiectasia mutated (*ATM*) gene regulates cellular responses to the DNA damage induced by ionizing radiation, an established breast carcinogen. It is unclear whether individuals who carry *ATM* mutations and are exposed to radiation are especially susceptible to radiation-induced breast cancer.

Study design

An international population-based case–control study nested within a cohort of 52 536 unilateral breast cancer survivors that investigated whether genetic variants in *ATM* play a clinically significant role in radiation-induced contralateral breast cancer.

Contribution

Women who carried rare *ATM* missense variants that are predicted to be deleterious and who were treated with radiation had an increased risk of developing contralateral breast cancer compared with nonirradiated women who carried the same predicted deleterious missense variant.

Implications

The increased risk of radiation-related contralateral breast cancer associated with specific *ATM* mutations may be an important factor in the selection of treatment for breast cancer for women who have a family history of ataxia-telangiectasia, a rare autosomal recessive disorder that arises from inactivating mutations in the *ATM* gene.

Limitations

There were few carriers of missense variants that were predicted to be deleterious in the study population, which limited the precision of the estimates.

From the Editors

in the risk of contralateral breast cancer associated with a radiation dose of 1 Gy or more to the contralateral breast among women younger than 40 years who survived at least 5 years after treatment compared with women who received no radiotherapy; however, no excess risk was observed in women older than 40 years (9). Nevertheless, the combined effect of these factors accounts for only a portion of the contralateral breast cancers that develop each year.

Patients with ataxia-telangiectasia (A-T), a rare autosomal recessive disorder that arises from inactivating mutations in the ataxia telangiectasia mutated (*ATM*) gene, display both cellular hypersensitivity to ionizing radiation and increased incidence of cancers (22–24). ATM, the protein product of the *ATM* gene, plays a central role in sensing and signaling the presence of DNA double-strand breaks that are induced by ionizing radiation (22,25,26). Upon activation by ionizing radiation, ATM phosphorylates a large number of proteins that control pathways that lead to cell cycle checkpoint arrest, DNA repair, or apoptosis. Among the hundreds of known substrates for ATM phosphorylation are the products of three genes that have been implicated in the etiology of contralateral breast cancer—*BRCA1*, *BRCA2*, and *CHEK2* (27,28)—which suggests that genetic variation in *ATM* might modify the activities of those proteins and thereby affect risk of cancer, particularly in the context of radiation exposure. Studies of A-T families have consistently reported an excess of female breast cancer among obligate heterozygous carriers of *ATM* mutations compared with noncarriers (29–36), including a study by Swift et al. (35) that demonstrated an association between self-reported radiation exposure and breast cancer among *ATM* obligate heterozygotes. However, the rarity and diversity of *ATM* mutations, coupled with the lack of well-documented radiation exposures among carriers, have limited the statistical power of case–control studies that evaluate either main (37–44) or radiation-dependent (35,45,46) associations between *ATM* mutations and risk of breast cancer.

It is unclear whether, and to what extent, genetic factors and radiation exposure, individually or via interaction, contribute to the development of radiation-induced contralateral breast cancer. A key unresolved question is whether individuals who carry *ATM* mutations and are exposed to radiation are especially susceptible to radiation-induced breast cancer. To address this question, we initiated the Women's Environmental Cancer and Radiation Epidemiology (WECARE) Study, a population-based nested case–control study designed specifically to evaluate interactions between genetic variation in genes such as *ATM* and radiation exposure in the etiology of breast cancer (47).

Subjects and Methods

Study Population

The WECARE Study is a multicenter population-based case–control study nested within a cohort of 52 536 women with histologically confirmed invasive breast cancer whose cancers were reported to one of four population-based cancer registries in the United States (the Los Angeles County Cancer Surveillance Program, the Cancer Surveillance System of the Fred Hutchinson Cancer Research Center [Seattle region], the State Health Registry of Iowa, and the Cancer Surveillance Program of Orange County/San Diego-Imperial Organization for Cancer Control [Orange County/San Diego]) or the nationwide Danish Breast Cancer Cooperative Group Registry. All participants were identified, recruited, and interviewed through these registries and were known to be cancer free during the first year after breast cancer diagnosis. The outcome of interest was subsequent primary cancer in the contralateral breast at least 1 year after the initial unilateral breast cancer diagnosis. The average interval between the first and second breast cancer diagnoses was 5 years (range = 1–16 years) (Supplementary Table 1, available online). The study design and the characteristics of the cohort have been described in detail (7,9,47).

The 708 case subjects were women with asynchronous contralateral breast cancer who met the following eligibility criteria: 1) was diagnosed between January 1, 1985, and December 31, 2000, with a first primary invasive breast cancer that had not spread beyond the regional lymph nodes at diagnosis and, at least 1 year after the first breast cancer diagnosis, with a second primary in situ (approximately 20% of contralateral breast cancers in this study) or invasive breast cancer in the contralateral breast; 2) resided in the same study reporting area for both diagnoses; 3) had no previous or intervening cancer diagnosis; 4) was younger than 55 years at

the time of diagnosis of the first primary breast cancer; 5) was alive at the time of contact for this study; and 6) was able to provide written informed consent, complete the interview, and provide a blood sample.

The 1397 control subjects were women with unilateral breast cancer who met the following criteria: 1) was diagnosed since January 1, 1985, with a first primary in situ or invasive breast cancer while residing in one of the study reporting areas; 2) resided during the "at-risk" interval (ie, the interval between the matched case subject's first and second breast cancer diagnoses) in the same cancer reporting area as when they were diagnosed with their breast cancer; 3) was never diagnosed (as of the reference date) with a second primary breast cancer or any other cancer; 4) was diagnosed with a first primary breast cancer before the age of 55 years; 5) was able to provide written informed consent, complete the interview, and provide a blood sample; and 6) had not had a prophylactic mastectomy of the contralateral breast (as of the reference date). Two control subjects were individually matched to each case subject on the year of birth (5-year strata), year of diagnosis (4-year strata), registry region, and race and/or ethnicity. The two control subjects were also countermatched on registry-reported radiotherapy (ever or never) to improve statistical efficiency. That is, each case subject and her two matched control subjects formed a triplet, wherein two members had received radiotherapy (according to the registry records) and one member had not. Countermatching was done to assure variation in radiation exposure within case–control sets while allowing for unbiased estimation of the main effects and interactions of interest. In this study, countermatching increased the precision of the radiation main effects and the *ATM* gene–radiation exposure interactions compared with random sampling of the same number of control subjects (47).

Data Sources

All WECARE Study participants were interviewed by telephone with the use of a questionnaire that focused on known and suspected risk factors for breast cancer (47). Medical records, pathology reports, and hospital charts were used to collect detailed information on all radiotherapy, chemotherapy, and hormonal therapy received for the treatment of primary breast cancer, metastases, recurrences, and benign conditions as well as tumor characteristics (47). Estimated absorbed radiation doses to various specific locations in the contralateral breast were reconstructed for each treatment regimen by using tissue-equivalent phantoms. Individual radiation doses were estimated in a blinded fashion with respect to case–control status and derived for the specific contralateral breast cancer locations and treatment regimen of the patient as previously described (9) and were available for 606 triplets.

Laboratory Methods

Detailed methods for the laboratory analyses and quality control used to generate the data analyzed in this study have been previously published (48,49). Briefly, DNA was prepared by phenol–chloroform extraction from blood samples that were collected at the time of interview. All coding exons (exons 4–65) of the *ATM* gene along with flanking intronic sequences ranging from 50 to 100 nucleotides in length were screened for sequence variation with the use of denaturing high-performance liquid chromatography as previously described (48,49). Amplicons that yielded sequence variants by denaturing high-performance liquid chromatography analysis were evaluated by direct nucleotide sequencing. The laboratories were blinded as to the case–control status of the samples and all matching information.

Statistical Analysis

To assess the association between *ATM* mutation carrier status and the risk of developing contralateral breast cancer, we estimated rate ratios (RRs) with corresponding 95% confidence intervals (CIs) by using univariate and multivariable conditional logistic regression models that included an "offset" term consisting of weights to adjust appropriately for the countermatched sampling (47,50). The multivariable models were adjusted for factors that were found to be statistically significantly associated with the risk of contralateral breast cancer in the univariate models and included the following: exact age at diagnosis of the first primary; age at menarche (<13 and ≥13 years); menopausal status (premenopausal, menopause before 45 years, and menopause at 45 years or older); number of full-term pregnancies (0, 1, 2, 3, or ≥4); family history of breast cancer among first-degree relative (yes or no); lobular histology and Surveillance, Epidemiology, and End Results summary stage [local and regional (47)] of the first primary; and treatment history (chemotherapy and hormonal and radiation therapy where indicated). Rate ratios that assess the effect of radiation dose on the risk of contralateral breast cancer were calculated by comparing, within each triplet, the dose received at the specific contralateral breast cancer location in the case subject with the dose received at the same breast location in her matched control subjects (51). Tests of homogeneity of the slopes of excess relative risk (ERR) per radiation dose in Gy across *ATM* variant subtypes (silent, splicing, missense, or truncating), age at diagnosis (<45 vs ≥45 years), and time since diagnosis (<5 vs ≥5 years) were performed by using likelihood ratio tests that compared the model that included a separate slope parameter for each age subgroup with one that included a single slope parameter. Cut points used for age at diagnosis and time since diagnosis were based on our prior work (9). Missing data indicators were used to account for missing covariate data (52). We calculated rate ratios that reflect two different referent groups; one rate ratio is relative to subjects with wild-type *ATM* who did not undergo radiotherapy, and the second is relative to subjects who carried the same class of mutation (eg, wild type, silent, missense, splicing, truncation, or common) and who did not receive radiotherapy. All analyses were conducted using the TPHREG procedure in SAS release 9.1 (SAS Institute, Inc, Cary, NC). All *P* values are two-sided, and those less than .05 were considered to be statistically significant.

Risk associated with carrying *ATM* variants was examined separately for common variants (ie, those carried by ≥1% of the WECARE Study participants) and for rare variants (ie, those with allele frequencies <1% in the WECARE Study population). Rare variants were classified on the basis of their predicted effect on the ATM protein as silent, missense, splicing, or truncating (Supplementary Table 2, available online). It should be noted that an individual subject may have more than one of these classes of

variants and that the variant classes are not mutually exclusive (eg, some splicing variants can result in truncation). Rare missense variants were further classified as to their predicted effect on ATM protein structure or function using the SIFT program (53), which predicts whether an amino acid substation will affect protein function based on a clustal alignment of available vertebrate ATM sequences. SIFT scores range from 0 to 1, and lower scores predict variants that are most likely to be deleterious. Based on the SIFT score, rare missense variants were therefore classified as tolerated or deleterious; variants with scores less than 0.05 are predicted to be deleterious. To summarize the variants over all 62 exons, we classified women whose ATM sequence differed from wild type at more than one position based on the sum of the SIFT scores of variants across all exons. If two rare missense variants occurred at a single exon, the lowest scoring variant was selected as the score for that exon. SIFT scores range from 0 to 1, and lower scores predict variants that are most likely to be deleterious. We compared the results of analyses using SIFT with those from another similar analytic program, PolyPhen (54), and found them to be equivalent. Therefore, we present here only the analyses that used SIFT scores. We also conducted analyses using the lowest-scoring single variant at any position (instead of the sum of the scores), and similar results were obtained. In each variant-specific model (and those using SIFT), the rate ratio was adjusted for the other variant types. To assess the effect of including in situ contralateral breast cancer, analyses were conducted including and excluding triplets in which the case subject was diagnosed with in situ contralateral breast cancer. Because those risk estimates were equivalent, all triplets were retained in the analyses presented here. Lastly, we conducted analyses by excluding BRCA1 and BRCA2 mutation carriers, and the risk estimates were again equivalent; therefore, the analyses presented here include the entire study population.

Results

We have previously reported individually on the association between *ATM* variants (49) and radiotherapy (9) and the risk of contralateral breast cancer in the WECARE Study population. In this study, we examined the interaction between these risk factors. Screening of the *ATM* gene in all 2105 women in the WECARE Study identified 240 unique variants, of which 147 were observed only a single time, 50 were observed two to five times, and 43 were observed six or more times. Our previous study found that when the 15 variants carried by more than 1% of WECARE Study participants were considered individually, four of these common variants were associated with a statistically significantly reduced risk of developing contralateral breast cancer (49). The remaining variants could only be considered for statistical analysis after grouping.

We first examined associations between *ATM* variants and the risk of asynchronous contralateral breast cancer. Rate ratios were calculated for *ATM* variants that were broadly classified by variant type and for missense variants that were classified using SIFT (53), for all case and control subjects, and for the case and control subjects who had location-specific radiation dose estimates (9) (Table 1). Overall, we observed no statistically significant elevated risk of contralateral breast cancer among women who carried any of the different types of *ATM* mutations compared with those who were wild type for *ATM*. Furthermore, there were no substantial differences between the rate ratios for women with and without location-specific dose estimates. For comparison with Renwick et al. (55), we also examined truncating mutations that included both premature termination codons and frameshift mutations that are known to be A-T causing and found a non-statistically significant elevated risk of contralateral breast cancer (RR = 2.0, 95% CI = 0.7 to 5.9).

Table 1. Rate ratio of asynchronous contralateral breast cancer associated with *ATM* gene mutation carrier status*

ATM variant classification	All case subjects (n = 708)	All control subjects (n = 1397)	RR (95% CI)	Case subjects with dose estimates† (n = 606)	Control subjects with dose estimates† (n = 1200)	RR (95% CI)
All variants, broadly classified						
Wild type	271	480	1.0 (referent)	223	418	1.0 (referent)
Silent	88	157	1.1 (0.7 to 1.5)	78	134	1.1 (0.8 to 1.6)
Missense	75	129	1.2 (0.8 to 1.7)	68	113	1.2 (0.8 to 1.8)
Splicing	4	16	0.6 (0.2 to 1.8)	4	14	0.7 (0.2 to 2.4)
Truncation	11	7	2.0 (0.7 to 5.9)	11	6	2.8 (0.9 to 8.9)
Common‡	355	778	0.8 (0.6 to 1.0)	308	655	0.8 (0.6 to 1.0)
Missense variants classified using SIFT§						
Wild type	271	480	1.0 (referent)	223	418	1.0 (referent)
Tolerated	36	72	0.9 (0.6 to 1.5)	31	66	0.9 (0.5 to 1.5)
Deleterious	39	56	1.4 (0.8 to 2.3)	37	46	1.7 (0.9 to 2.9)

* The multivariable models were adjusted for factors found to be statistically significantly associated with contralateral breast cancer in the univariate models and those known to be associated with breast cancer, including the following: exact age at diagnosis of first primary breast cancer, age at menarche (<13 or ≥13 years), menopausal status (premenopausal and age at menopause <45 or ≥45 years), number of full-term pregnancies (0, 1, 2, 3, or ≥4), family history of breast cancer among first-degree relative (yes or no), lobular histology (yes or no) and Surveillance, Epidemiology, and End Results summary stage (47) (local or regional) of the first primary, and treatment history (chemotherapy or hormonal therapy and radiation where indicated). CI = confidence interval; RR = rate ratio.

† Case subjects and matched control subjects for whom there were location-specific dose estimates.

‡ *ATM* variants carried by 1% or more of the WECARE Study participants.

§ Variants with normalized probabilities less than .05 are predicted to be deleterious, whereas those with probabilities equal to or greater than .05 are predicted to be tolerated. Results for missense variants are adjusted for other variants. One control subject carried only one variant that lacked a SIFT classification.

Among women exposed to radiation, the mean dose received to the contralateral breast was 1.2 Gy (SD = 0.7). For women who carried rare *ATM* missense variants, those with radiation exposure levels of 1.0 Gy or higher had an increased risk of contralateral breast cancer compared with women who were wild type for *ATM* and unexposed to radiation (RR = 2.0, 95% CI = 1.1 to 3.9); the dose–response trend was statistically significant (ERR/Gy = 1.3, 95% CI = 0.1 to 3.9; P_{trend} = .017) (Table 2). Among women who carried an *ATM* missense variant that was predicted to be deleterious, those who were exposed to radiation had a statistically significantly higher risk of contralateral breast cancer compared with unexposed women who were wild type for *ATM* (0.01–0.99 Gy: RR = 2.8, 95% CI = 1.2 to 6.5; ≥1.0 Gy: RR = 3.3, 95% CI = 1.4 to 8.0) or unexposed women who carried predicted deleterious missense variants (0.01–0.99 Gy: RR = 5.3, 95% CI = 1.6 to 17.3; ≥1.0 Gy: RR = 5.8, 95% CI = 1.8 to 19.0; ERR/Gy = 2.6, 95% CI = 0.0 to 10.6; P_{trend} = .044). These trends were not evident among women who carried other types of *ATM* variants.

Analyses assessing variation by age at and time since breast cancer diagnosis using all case and control subjects, with radiotherapy stratified as ever or never, were conducted to provide more stable estimates (Table 3). Among women who carried *ATM* missense variants that were predicted to be deleterious, the rate ratio for ever vs never use of radiotherapy appeared to be greater for women younger than 45 years at diagnosis (RR = 10.4, 95% CI = 2.3 to 47.2) than for older women (RR = 2.4, 95% CI = 0.6 to 9.5).

Table 2. Rate ratio of developing contralateral breast cancer by radiation exposure and *ATM* gene mutation carrier status*

ATM variants	Radiation exposure, Gy†	Case subjects (n = 606)	Control subjects (n = 1200)	RR‡ (95% CI)	RR§ (95% CI)	ERR/Gy (95% CI)
All variants, broadly classified						
Wild type	0	112	72	1.0 (referent)	1.0 (referent)	
	0.01–0.99	57	177	1.1 (0.7 to 1.6)	1.1 (0.7 to 1.6)	
	≥1.0	54	169	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	0.0 (<<0 to 0.3)
Silent	0	38	29	1.1 (0.6 to 2.0)	1.0 (referent)	
	0.01–0.99	25	59	1.3 (0.7 to 2.2)	1.1 (0.5 to 2.4)	
	≥1.0	15	46	1.0 (0.5 to 2.0)	0.9 (0.4 to 2.2)	0.2 (−0.3 to 1.3)
Missense	0	26	30	0.6 (0.3 to 1.1)	1.0 (referent)	
	0.01–0.99	21	45	1.7 (0.9 to 3.1)	2.7 (1.2 to 6.4)	
	≥1.0	21	38	2.0 (1.1 to 3.9)	3.3 (1.4 to 8.0)	1.3 (0.1 to 3.9)
Splicing	0	0	2	—	1.0 (referent)	
	0.01–0.99	3	6	1.5 (0.4 to 6.5)	—	
	≥1.0	1	6	0.4 (0.0 to 3.6)	—	—
Truncation	0	6	3	1.6 (0.3 to 8.6)	1.0 (referent)	
	0.01–0.99	3	3	2.9 (0.5 to 16.3)	1.7 (0.2 to 19.3)	2.5 (−0.4 to 36.3)
	≥1.0	2	0	—	—	
Common‖	0	154	126	0.8 (0.6 to 1.2)	1.0 (referent)	
	0.01–0.99	84	308	0.8 (0.5 to 1.1)	0.9 (0.6 to 1.3)	
	≥1.0	70	221	0.9 (0.6 to 1.4)	1.1 (0.7 to 1.6)	0.0 (−0.2 to 0.3)
Missense variants classified by SIFT¶						
Wild type	0	112	72	1.0 (referent)	1.0 (referent)	
	0.01–0.99	57	177	1.1 (0.7 to 1.6)	1.1 (0.7 to 1.6)	
	≥1.0	54	169	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	0.0 (<<0 to 0.3)
Tolerated	0	12	16	0.7 (0.3 to 1.7)	1.0 (referent)	
	0.01–0.99	9	27	1.1 (0.4 to 2.7)	1.6 (0.5 to 5.2)	
	≥1.0	10	23	1.3 (0.6 to 3.2)	1.8 (0.6 to 5.8)	0.8 (−0.1 to 3.6)
Deleterious	0	14	14	0.6 (0.2 to 1.3)	1.0 (referent)	
	0.01–0.99	12	17	2.8 (1.2 to 6.5)	5.3 (1.6 to 17.3)	
	≥1.0	11	15	3.3 (1.4 to 8.0)	5.8 (1.8 to 19.0)	2.6 (0.0 to 10.6)

* Case and matched control subjects were women with estimates of the reconstructed location-specific dose received to contralateral breast during radiotherapy. — = no estimate; CI = confidence interval; ERR = excess relative risk; RR = rate ratio.

† Defined as reconstructed quadrant dose received to contralateral breast during radiotherapy; 0.01–0.99 Gy category: range = 0.02–0.99, mean = 0.6 (SD = 0.2); ≥1.0 Gy category: range = 1.1–6.2, mean = 1.7 (SD = 0.6).

‡ The baseline comparison group is wild type and unexposed. Conditional logistic regression models are fully adjusted for factors found to be statistically significantly associated with contralateral breast cancer in the univariate models and those known to be associated with breast cancer, including the following: exact age at diagnosis of first primary breast cancer, age at menarche (<13 or ≥13 years), menopausal status (premenopausal and age at menopause <45 or ≥45 years), number of full-term pregnancies (0, 1, 2, 3, or ≥4), family history of breast cancer among first-degree relative (yes or no), lobular histology (yes or no) and stage (local or regional) of the first primary, and treatment history (chemotherapy or hormonal therapy and radiation where indicated).

§ The comparison group in this model is unexposed carriers of the same type of mutation. Multivariable conditional logistic regression was used to adjust for the same factors as above.

‖ *ATM* variants carried by 1% or more of the WECARE Study participants.

¶ Variants with normalized probabilities less than .05 are predicted to be deleterious, whereas those with probabilities equal to or greater than .05 are predicted to be tolerated. Results for missense variants are adjusted for other variants. One control subject carried only one variant that lacked a SIFT classification.

Table 3. Joint effects of *ATM* gene mutation carrier status, radiation exposure, and risk of developing asynchronous contralateral breast cancer according to age at and time since breast cancer diagnosis*

ATM variants and age and latency factors		Radiation exposure	Case subjects (n = 708)	Control subjects (n = 1397)	RR (95% CI)	RR (95% CI)	ERR/Gy† (95% CI)
Missense variants classified using SIFT‡							
Wild type		Never	142	82	1.0 (referent)	1.0 (referent)	
		Ever	129	398	0.9 (0.6 to 1.3)	0.9 (0.6 to 1.3)	0.0 (<<0 to 0.3)
Tolerated		Never	17	17	0.8 (0.5 to 1.0)	1.0 (referent)	
		Ever	19	55	0.9 (0.5 to 1.8)	1.2 (0.5 to 3.1)	0.8 (−0.1 to 3.6)
Deleterious		Never	14	15	0.4 (0.2 to 1.0)	1.0 (referent)	
		Ever	25	41	2.1 (1.1 to 3.8)	5.0 (1.8 to 13.3)	2.6 (0.0 to 10.6)
Age at diagnosis and SIFT classification, y							
23–44	Wild type	Never	62	36	1.0 (referent)	1.0 (referent)	
		Ever	60	181	0.9 (0.6 to 1.4)	0.9 (0.6 to 1.4)	0.0 (<<0 to 0.5)
	Tolerated	Never	7	3	2.0 (0.4 to 9.3)	1.0 (referent)	
		Ever	9	25	0.8 (0.3 to 2.1)	0.4 (0.1 to 2.4)	0.7 (<<0 to 4.6)
	Deleterious	Never	5	10	0.2 (0.1 to 0.7)	1.0 (referent)	
		Ever	10	16	2.0 (0.8 to 5.0)	10.4 (2.3 to 47.2)	4.9 (−0.1 to 24.9)
≥45	Wild type	Never	80	46	1.0 (referent)	1.0 (referent)	
		Ever	69	217	0.9 (0.6 to 1.3)	0.9 (0.6 to 1.3)	−0.0 (<<0 to 0.4)
	Tolerated	Never	10	14	0.5 (0.2 to 1.3)	1.0 (referent)	
		Ever	10	30	1.0 (0.4 to 2.3)	1.9 (0.6 to 6.2)	0.8 (−0.1 to 4.5)
	Deleterious	Never	9	5	0.9 (0.2 to 3.0)	1.0 (referent)	
		Ever	15	25	2.0 (1.0 to 4.4)	2.4 (0.6 to 9.5)	2.2 (−0.0 to 9.6)
Time since diagnosis and SIFT classification, y							
1–4	Wild type	Never	81	48	1.0 (referent)	1.0 (referent)	
		Ever	77	238	0.8 (0.6 to 1.2)	0.8 (0.6 to 1.2)	−0.1 (<<0 to 0.3)
	Tolerated	Never	11	11	0.8 (0.3 to 2.1)	1.0 (referent)	
		Ever	14	33	1.1 (0.5 to 2.4)	1.4 (0.5 to 4.4)	1.2 (−0.2 to 5.4)
	Deleterious	Never	8	8	0.3 (0.1 to 1.1)	1.0 (referent)	
		Ever	14	23	1.8 (0.8 to 3.9)	5.2 (1.4 to 18.8)	1.9 (−0.1 to 9.1)
≥5	Wild type	Never	61	34	1.0 (referent)	1.0 (referent)	
		Ever	52	160	1.0 (0.7 to 1.7)	1.0 (0.7 to 1.7)	0.2 (<<0 to 0.8)
	Tolerated	Never	6	6	0.8 (0.2 to 2.7)	1.0 (referent)	
		Ever	5	22	0.5 (0.2 to 1.8)	0.7 (0.1 to 4.0)	0.3 (−0.2 to 3.1)
	Deleterious	Never	6	7	0.6 (0.2 to 2.2)	1.0 (referent)	
		Ever	11	18	2.5 (1.0 to 6.1)	4.3 (0.9 to 19.9)	4.3 (0.1 to 20.7)

* The multivariable models were adjusted for factors found to be statistically significantly associated with contralateral breast cancer in the univariate models and those known to be associated with breast cancer, including the following: exact age at diagnosis of first primary breast cancer, age at menarche (<13 or ≥13 years), menopausal status (premenopausal and age at menopause <45 or ≥45 years), number of full-term pregnancies (0, 1, 2, 3, or ≥4), family history of breast cancer among first-degree relative (yes or no), lobular histology (yes or no) and stage (local or regional) of the first primary, and treatment history (chemotherapy or hormonal therapy and radiation where indicated). CI = confidence interval; ERR = excess relative risk; RR = rate ratio.

† Dose is based on 606 triplets for whom there were dose estimates made.

‡ Variants with normalized probabilities less than .05 are predicted to be deleterious, whereas those with probabilities equal to or greater than .05 are predicted to be tolerated. Results for missense variants are adjusted for other variants. One control subject carried only one variant that lacked a SIFT classification.

However, the 95% confidence intervals on the subgroup-specific linear slope estimates were wide, and the excess relative risks per radiation dose did not differ statistically significantly from each other (ERR/Gy for women younger than 45 years = 4.9 [95% CI = −0.1 to 24.9] and for women 45 years or older = 2.2 [95% CI = −0.0 to 9.6]). Furthermore, the large rate ratio in the younger age group relative to unexposed carriers of deleterious variants must be interpreted with caution because the rate ratio for women who carried such variants compared with radiation-unexposed women who were wild type for *ATM* was 0.2 (95% CI = 0.1 to 0.7). Similarly, among women who carried predicted deleterious missense variants, we observed a steeper radiation dose slope for those with a longer latency (≥5 years) compared with those with a shorter latency (ERR/Gy: 4.3 [95% CI = 0.1 to 20.7] vs 1.9 [95% CI = −0.1 to 9.1], respectively), but this difference was also not statistically significant. Furthermore, the rate ratios for the ever vs never radiotherapy comparisons did not differ appreciably by time since first breast cancer diagnosis. The data were too sparse to obtain statistically meaningful results for analyses of the joint effects of *ATM* variant carrier status, age at diagnosis, and latency.

Discussion

Our findings suggest that *ATM* genetic variation and radiation exposure have joint etiologic roles in contralateral breast cancer in a small fraction of women who carry specific types of *ATM* gene

variants. A current major focus of many epidemiological studies is to characterize the interactions between genetic and environmental factors (56). Although many environmental factors are not easily defined or quantified, radiation exposures in radiotherapy and in certain other settings are an exception. One advantage of our nested population-based case–control study is that it was specifically designed to maximize the statistical power to address the hypothesis that women who carry *ATM* gene mutations and who were exposed to radiation are at increased risk of developing radiation-induced breast cancer. In general, the low prevalence of relatively deleterious genetic abnormalities and the low levels of radiation present in most environments limit the informativeness of studies of the interaction of *ATM* gene and radiation exposure conducted in the general population (12,43). By restricting our analysis to a population of genetically high-risk women with bilateral breast cancer who were exposed to substantial and quantifiable levels of ionizing radiation, coupled with a full characterization of the *ATM* gene in the study population, we enhanced our ability to detect possible gene–radiation interactions. This study builds on our previously reported work demonstrating that the risk of developing contralateral breast cancer was positively associated with the dose of radiation received and inversely associated with the woman's age at radiation exposure (9) and that *ATM* gene carrier status alone was not associated with contralateral breast cancer (49). However, in this study, we found that among carriers of *ATM* missense mutations who were treated with radiotherapy, the rate ratio of radiation-induced contralateral breast cancer was twofold compared with women who were not treated with radiation and who were wild type for *ATM*. This effect was dose dependent, and the risk of contralateral breast cancer was greater when *ATM* missense variants predicted to be deleterious were considered; the effect was weakly time and age dependent.

Our finding of a possible interaction of *ATM* variation and radiation exposure in breast cancer etiology is plausible when considered in light of both the existing literature on the role of *ATM* in risk of breast cancer and our previous examination of the main effects of *ATM* variants in the WECARE Study population. For example, there is evidence from multiple epidemiological studies of A-T families that heterozygosity for A-T–causing mutations in *ATM* is associated with an increased risk of breast cancer (29–36). These mutations, which predominate among patients with A-T (A-T Mutation Database, www.LOVD.nl/ATM), are primarily those predicted to prematurely truncate the ATM protein. Although A-T–causing mutations are relatively rare in the general population, and even in high-risk breast cancer populations, they are associated with the risk of breast cancer in families ascertained for multiple breast cancer cases (55). It seems unlikely that carriers of these rare mutations would coincidentally be enriched for radiation exposure. Thus, this risk appears to be a main effect of the presence of these inactivating ATM mutations.

ATM is a master regulator of cellular pathways that mediate responses to the most deleterious form of DNA damage induced by ionizing radiation, DNA double-strand breaks. Variants in the *ATM* gene that might interact with ionizing radiation need not be restricted to the rare protein-truncating mutations that cause A-T, which is precisely what we observed in this study. The risk of radiation-associated second primary breast cancers was most strongly associated with rare missense variants in *ATM* that are predicted to have deleterious effects on protein structure that do not involve protein truncation. How might these two different mechanisms whereby ATM affects risk of breast cancer be reconciled? We propose that in carriers of truncating mutations, *ATM* acts as a classic tumor suppressor gene and that loss of heterozygosity or epigenetic silencing of the normal allele occurs in tumor tissue. The loss or silencing of the normal allele could be induced by exposure to a mutagen like ionizing radiation but would not be dependent on such exposure. For carriers of the rare missense variants reported here, we propose that these variant likely act by dominant interference. Thus, the presence of rare missense variants effectively reduces the level of *ATM* activity below the 50% level expected in carriers of truncating mutations, which results in susceptibility to radiation-induced tumorigenesis. A key prediction of this model is that nontumor tissue, such as that in the contralateral breast, would have increased radiosensitivity in carriers of missense variants but not in carriers of truncating variants. Therefore, exposure to radiation scatter doses greater than 1 Gy may result in greater than normal amounts of DNA damage in cells of the contralateral breast, particularly in carriers of missense variants. If these cells survive, the radiation-induced DNA damage may increase the chance that a tumor will subsequently develop in the contralateral breast.

Our study included 2105 women with breast cancer who were screened for variants in all 62 coding exons in the *ATM* gene. However, our ability to detect associations between individual variants and the risk of contralateral breast cancer was limited because many of the unique non-silent *ATM* variants occurred infrequently. Therefore, we used different approaches for grouping the variants in biologically meaningful ways. First, we examined variants that were broadly classified according to the effect of the DNA change on the amino acid sequence and found no indication of an interaction with radiation exposure, including among women who carried common variants that we had previously reported were associated with a decreased risk of contralateral breast cancer (49). Second, we also examined truncating mutations that included both premature termination codons and frameshift mutations that are known to be A-T causing. In our series, however, less than 1% of the variants detected were truncating mutations, which limited our ability to examine their interaction with radiation. Our overall findings among women with a truncating or A-T–causing mutation (RR = 2.0, 95% CI = 0.7 to 5.9) are not inconsistent with the findings of Renwick et al. (55), who screened 443 breast cancer case subjects from multiple-case families and 521 control subjects for *ATM* mutations. They found a statistically significant association between *ATM* mutations that cause A-T and breast cancer (RR = 2.4, 95% CI = 1.5 to 3.8). However, they did not examine the effect of radiation, and the number of subjects with bilateral cancers was small, making a direct comparison of the results difficult.

A major strength of our study is that we had accurate estimates of radiation doses to tumor sites and simultaneously conducted a comprehensive characterization of variants in the *ATM* gene to highlight a gene by environment interaction. Nevertheless, we detected few carriers of missense variants that were predicted to be deleterious by SIFT, which limited the precision of our estimates.

We observed large rate ratios associated with *ATM* missense variants and radiation exposures greater than 1 Gy; however, the estimates of radiation-induced risk for all missense variants (ERR/Gy = 1.3, 95% CI = 0.1 to 3.9) and for the predicted deleterious missense variants (ERR/Gy = 2.6, 95% CI = 0.0 to 10.6) are statistically compatible with those reported in radiation studies of other populations, such as atomic bomb survivors (ERR/Gy = 1.49, 95% CI = 1.17 to 1.85) and a pooled analysis of eight cohort studies (ERR/Gy = 0.9, 95% CI = 0.7 to 1.0) (57). Furthermore, although young age at breast cancer diagnosis and long latency are hallmarks of radiation-induced breast cancer (57), the strong radiation effect we observed in women younger than 45 years at diagnosis who carried deleterious mutations had great uncertainty because of these small numbers. Therefore, although the increased risk of radiation-related contralateral breast cancer associated with specific *ATM* mutations is not an important factor in the selection of treatment for breast cancer for most women, it might warrant consideration in the rare instances where a woman has a family history of A-T. Further epidemiological study of the role of *ATM* missense mutations among women diagnosed with breast cancer before the age of 45 years who were treated with radiation and developed contralateral breast cancer after a long latency period is necessary to better estimate these risks.

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