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Brief Communication

Radiation Treatment, *ATM*, *BRCA1/2*, and *CHEK2**1100delC Pathogenic Variants and Risk of Contralateral Breast Cancer

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Abstract

Whether radiation therapy (RT) affects contralateral breast cancer (CBC) risk in women with pathogenic germline variants in moderate- to high-penetrance breast cancer–associated genes is unknown. In a population-based case-control study, we examined the association between RT; variants in *ATM*, *BRCA1/2*, or *CHEK2**1100delC; and CBC risk. We analyzed 708 cases of women with CBC and 1399 controls with unilateral breast cancer, all diagnosed with first invasive breast cancer between 1985 and 2000 and aged younger than 55 years at diagnosis and screened for variants in breast cancer–associated genes. Rate ratios (RR) and 95% confidence intervals (CIs) were estimated using multivariable conditional logistic regression. RT did not modify the association between known pathogenic variants and CBC risk (eg, *BRCA1/2* pathogenic variant carriers without RT: RR = 3.52, 95% CI = 1.76 to 7.01; *BRCA1/2* pathogenic variant carriers with RT: RR = 4.46, 95% CI = 2.96 to 6.71), suggesting that modifying RT plans for young women with breast cancer is unwarranted. Rare *ATM* missense variants, not currently identified as pathogenic, were associated with increased risk of RT-associated CBC (carriers of *ATM* rare missense variants of uncertain significance without RT: RR = 0.38, 95% CI = 0.09 to 1.55; carriers of *ATM* rare missense variants of uncertain significance with RT: RR = 2.98, 95% CI = 1.31 to 6.80). Further mechanistic studies will aid clinical decision-making related to RT.

Radiation therapy (RT) improves breast cancer survival, but women treated with RT for a first primary breast cancer are at an increased risk of developing contralateral breast cancer (CBC) (1,2), making accurate estimation of late effects paramount. Pathogenic variants in several genes whose products regulate the cellular response to DNA damage induced by ionizing radiation (eg, *BRCA1*, *BRCA2*, *ATM*, *PALB2*, and *CHEK2*) are associated with increased CBC risk. However, the extent to which these pathogenic variants, given their potential impact on DNA damage responses, may interact with ionizing radiation exposure occurring during RT is unknown but of clinical concern. In this study, we examine the interaction of RT and genetic variants in the *ATM*, *BRCA1/2*, and *CHEK2* genes and its effect on cumulative CBC risk.

The Women's Environmental Cancer and Radiation Epidemiology (WECARE) study is a population-based study of 708 CBC cases and 1399 matched controls with unilateral breast cancer, all diagnosed with first invasive breast cancer between 1985 and 2000 and younger than 55 years of age at diagnosis. Eligible women were identified through 5 population-based

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Table 1. Mutation status and radiation therapy for first primary breast cancer and association with CBC risk in the WECARE Study

Mutation	Carriership and radiation	CBC No.* (%)	UBC† No.* (weighted %‡)	Adjusted RR (95% CI§)	P§	P_{het}§,‖
ATM pathogenic/likely pathogenic	Noncarrier	694 (98.0)	1382 (98.4)	1.00 (Referent)		
	Carrier	14 (2.0)	15 (1.6)	1.68 (0.75 to 3.76)	.20	N/A
BRCA1/2¶	Noncarrier	596 (84.5)	1322 (94.9)	1.00 (Referent)		
	Carrier	109 (15.5)	76 (5.1)	4.23 (2.92 to 6.12)	<.001	N/A
*CHEK2**1100delC	Noncarrier	701 (99.0)	1385 (99.1)	1.00 (Referent)		
	Carrier	7 (1.0)	10 (0.9)	2.17 (0.72 to 6.55)	.17	N/A
ATM rare missense VUS#	Noncarrier	694 (98.0)	1373 (98.3)	1.00 (Referent)		
	Carrier	14 (2.0)	24 (1.7)	1.67 (0.78 to 3.58)	.19	N/A
ATM pathogenic/likely pathogenic	Noncarrier	694 (98.0)	1382 (98.4)	1.00 (Referent)		
	Carrier without RT	7 (1.0)	4 (0.8)	1.33 (0.36 to 4.99)	.67	.67
	Carrier with RT	7 (1.0)	11 (0.7)	1.91 (0.71 to 5.12)	.20	
BRCA1/2¶	Noncarrier	596 (84.5)	1322 (94.9)	1.00 (Referent)		
	Carrier without RT	46 (6.5)	12 (2.2)	3.52 (1.76 to 7.01)	<.001	.55
	Carrier with RT	63 (8.9)	64 (3.0)	4.46 (2.96 to 6.71)	<.001	
*CHEK2**1100delC	Noncarrier	701 (99.0)	1385 (99.1)	1.00 (Referent)		
	Carrier without RT	2 (0.3)	2 (0.4)	0.76 (0.09 to 6.29)	.80	.28
	Carrier with RT	5 (0.7)	8 (0.5)	3.00 (0.90 to 9.99)	.07	
ATM rare missense VUS#	Noncarrier	694 (98.0)	1373 (98.3)	1.00 (Referent)		
	Carrier without RT	3 (0.4)	8 (1.1)	0.38 (0.09 to 1.55)	.18	.008
	Carrier with RT	11 (1.6)	16 (0.6)	2.98 (1.31 to 6.80)	.009	

*Two women with UBC were not screened for *ATM* variants. Three women with CBC and one woman with UBC were not screened for *BRCA1/2* mutations. Four women with UBC were not screened for *CHEK2**1100delC. CBC = contralateral breast cancer; CI = confidence interval; RR = rate ratio; RT = radiation therapy; UBC = unilateral breast cancer; VUS = variants of uncertain significance; WECARE = Women's Environmental Cancer and Radiation Epidemiology.

†UBC controls must not have undergone prophylactic mastectomy of the contralateral breast prior to their reference date. The reference date for controls was defined by adding the interval between the first breast cancer and CBC for the matched case to the date of UBC for the control.

‡Weighted proportions to reflect the countermatched study design of WECARE.(3) Proportions cannot be directly calculated from numbers of UBC controls. Ratios cannot be directly calculated from numbers in this table.

§Adjusted for age at first primary breast cancer diagnosis, age at menarche, age at menopause, chemotherapy and hormonal therapy, histology of the first primary breast cancer, stage of the first primary breast cancer, and number of full-term pregnancies. *ATM* and *CHEK2**1100delC results are also adjusted for *BRCA1/2* mutation carrier status.

‖Two-sided *P* values for heterogeneity of RT status within carriers of a specific mutation. A two-sided *P* value for heterogeneity of RT status with *ATM* rare missense VUS carriers was also calculated using a permutation test. This resulted in a two-sided *P* value of .009.

¶Some *BRCA1/2* variants previously classified as VUS were updated as pathogenic mutations since our 2010 publication.(4)

#All of the *ATM* rare missense VUS identified had allele frequencies of <0.0001 in the genome aggregation database.

cancer registries in Denmark and the United States (Iowa state, Los Angeles County and the Orange County–San Diego regions of California, and western Washington state). The study protocol was approved by the institutional review board at each site and the Denmark ethics committee, and all participants provided written informed consent. Inclusion criteria (1,3,4), individual matching (1,3,4) of participants on birth and diagnosis years, registry and race and ethnicity, countermatching of participants on radiation (1), and mutation screening of *BRCA1/2* (4), *ATM* (5), and *CHEK2**1100delC (6) are described elsewhere.

ATM variants were classified as pathogenic or likely pathogenic (PLP) or of uncertain significance using ClinVar (7), a clinical genetics database with 5 levels of function: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (Supplementary Table 1, available online). Rare *ATM* or *BRCA1/2* missense variants were defined as those resulting in a single amino acid substitution with a frequency of less than 0.01 in the WECARE study and for which no homozygous individuals were listed in the genome aggregation database (8). Although not used as an explicit threshold, in the genome aggregation database, these variants all had allele frequencies less than 0.0001. All were classified as variants of uncertain significance (VUS) in ClinVar.

Multivariable rate ratios (RR) and 95% confidence intervals (CI) were estimated as previously described (4,6) by fitting conditional logistic regression models adjusted for age at first primary breast cancer, age at menarche, age at menopause, chemotherapy and hormonal therapy, histology of the first primary breast cancer, stage of the first primary breast cancer, number of full-term pregnancies, and for *ATM* and *CHEK2**1100delC models, known *BRCA1/2* pathogenic mutation status. Heterogeneity tests were performed using the likelihood ratio test for 2 nested regression models. Five- and 10-year cumulative risks of CBC by RT and mutation status were estimated by combining frequencies and estimates from the WECARE study with population-based incidence rates from the Surveillance, Epidemiology and End Results (SEER) Program (9) using previously described methodology (4). Analyses were performed in SAS v9.4. All statistical tests were two-sided, and a *P* of less than .05 was considered statistically significant.

Women with pathogenic variants in *BRCA1/2* had an increased CBC risk (RR = 4.23, 95% CI = 2.92 to 6.12) (Table 1) and a 10-year cumulative CBC risk of 19.3% (95% CI = 13.2% to 28.2%) (Table 2). This statistically significant increase in cumulative CBC risk for carriers of *BRCA1/2* pathogenic variants remained regardless of treatment with RT (*BRCA1/2* pathogenic variant carriers without RT: RR = 3.52, 95% CI = 1.76 to 7.01; *BRCA1/2* pathogenic variant carriers with RT: RR = 4.46, 95% CI = 2.96 to 6.71). Carriers of an *ATM* PLP variant, an *ATM* rare missense

Table 2. Cumulative risks of CBC by mutation status and radiation therapy for first primary breast cancer

Mutation	Carriership and radiation	5-year CBC risk, % (95% CI)	10-year CBC risk, % (95% CI)
SEER benchmark	—	2.9	5.8
ATM pathogenic/likely pathogenic	Carrier	4.8 (2.1 to 10.7)	9.3 (4.1 to 20.7)
*BRCA1/2**	Carrier	10.2 (7.0 to 14.9)	19.3 (13.2 to 28.2)
*CHEK2**1100delC	Carrier	6.1 (2.0 to 18.5)	11.8 (3.9 to 35.8)
ATM rare missense VUS†	Carrier	4.7 (2.2 to 10.2)	9.2 (4.3 to 19.8)
ATM pathogenic/likely pathogenic	Carrier without RT	3.8 (1.0 to 14.2)	7.4 (2.0 to 27.8)
	Carrier with RT	5.4 (2.0 to 14.5)	10.5 (3.9 to 28.2)
*BRCA1/2**	Carrier without RT	8.6 (4.3 to 17.1)	16.4 (8.2 to 32.7)
	Carrier with RT	10.7 (7.1 to 16.3)	20.3 (13.4 to 30.7)
*CHEK2**1100delC	Carrier without RT	2.2 (0.3 to 18.0)	4.3 (0.5 to 35.5)
	Carrier with RT	8.4 (2.5 to 28.0)	16.0 (4.8 to 53.5)
ATM rare missense VUS†	Carrier without RT	1.1 (0.3 to 4.5)	2.2 (0.5 to 9.0)
	Carrier with RT	8.4 (3.7 to 19.1)	16.0 (7.0 to 36.5)

*Some *BRCA1/2* variants previously classified as VUS were updated as pathogenic mutations since our 2010 publication. (4) — = SEER does not provide reliable treatment information or any information on mutation carrier status; CBC = contralateral breast cancer; CI = confidence interval; RT = radiation therapy; SEER = Surveillance, Epidemiology, and End Results; VUS = variants of uncertain significance.
†All of the *ATM* rare missense VUS identified had allele frequencies of < 0.0001 in the genome aggregation database.

VUS, or the *CHEK2**1100delC allele did not have a statistically significantly elevated CBC risk (Table 1), and therefore, cumulative CBC risks were not different from SEER benchmark risks (Table 2). RT statistically significantly modified CBC risk by *ATM* rare missense VUS status (*ATM* rare missense VUS carriers without RT: RR = 0.38, 95% CI = 0.09 to 1.55; *ATM* rare missense VUS carriers with RT: RR = 2.98, 95% CI = 1.31 to 6.80; $P_{heterogeneity} = .008$) but not by *BRCA1/2* rare missense VUS (data not shown) (Table 1). The cumulative CBC risks for carriers of *ATM* rare missense VUS were statistically significantly elevated only among those who received RT for their first primary breast cancer (10-year risk = 16.0%, 95% CI = 7.0% to 36.5%).

Next-generation sequencing and multigene panel testing for hereditary cancer genes have been integrated into clinical practice, creating both opportunities and challenges with regard to how to best incorporate this information into treatment decisions. To our knowledge, this is the first study to estimate cumulative CBC risks for women carrying pathogenic variants in breast cancer risk genes who received RT. Whereas carriers of PLP variants in *ATM*, pathogenic variants in *BRCA1/2*, or *CHEK2**1100delC were not more likely to develop CBC after RT, we unexpectedly found that women with *ATM* rare missense VUS had an increased CBC risk compared with the SEER benchmark risk.

Our findings extend prior but smaller, less comprehensive studies reporting no demonstrable elevated CBC risk among *BRCA1/2* mutation carriers treated with RT (10,11). We found a nonstatistically significant, but elevated, CBC risk for *CHEK2**1100delC carriers treated with RT. This finding is consistent with the only prior study of CBC risk in *CHEK2* mutation carriers treated with RT (12). Although our population-based study was large with long-term follow-up, the relatively small number of carriers with pathogenic variants precluded risk estimation within certain subgroups, including by RT-absorbed dose to the contralateral breast and variant-specific risks for *BRCA1/2* or *ATM*.

The present study extends knowledge of the relationship between allelic variation in *ATM* and CBC risk, showing that some individual rare *ATM* missense variants, currently classified as VUS in ClinVar, act jointly with RT to substantially increase cumulative CBC risks. Previous work has proposed a distinction between null alleles (ie, PLP) at *ATM* that make little or no detectable protein and missense alleles that potentially produce a defective protein that could be incorporated into cellular complexes, disrupting their function (13). Our data, although preliminary, suggest that this distinction may have functional importance that is not discernable from current definitions of pathogenicity or the positions of variants in the ATM protein (Supplementary Figure 1, available online). Understanding the mechanism(s) of action of these *ATM* variants is necessary before this observation can inform clinical practice.

In summary, we report that women who carry *ATM* variants classified as PLP in ClinVar, pathogenic mutations in *BRCA1/2*, or *CHEK2**1100delC may not be at increased risk of radiation-associated CBC. The increased RT-related risk for women with *ATM* rare missense VUS highlights the need for improved tools and approaches to resolve the functional impact of such variants, their interaction with RT, and subsequent CBC risk.

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