Research article

No evidence for association of ataxia-telangiectasia mutated gene T2119C and C3161G amino acid substitution variants with risk of breast cancer

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Received: 5 June 2002
Revisions requested: 23 July 2002
Revisions received: 30 July 2002
Accepted: 31 July 2002
Published: 21 August 2002

Breast Cancer Res 2002, **4**:R15

(Print ISSN 1465-5411; Online ISSN 1465-542X)

Abstract

Background: There is evidence that certain mutations in the double-strand break repair pathway ataxia-telangiectasia mutated gene act in a dominant-negative manner to increase the risk of breast cancer. There are also some reports to suggest that the amino acid substitution variants T2119C Ser707Pro and C3161G Pro1054Arg may be associated with breast cancer risk. We investigate the breast cancer risk associated with these two nonconservative amino acid substitution variants using a large Australian population-based case–control study.

Methods: The polymorphisms were genotyped in more than 1300 cases and 600 controls using 5′ exonuclease assays. Case–control analyses and genotype distributions were compared by logistic regression.

Results: The 2119C variant was rare, occurring at frequencies of 1.4 and 1.3% in cases and controls, respectively ($P = 0.8$). There was no difference in genotype distribution between cases and controls ($P = 0.8$), and the TC genotype was not associated with increased risk of breast cancer (adjusted odds ratio = 1.08, 95% confidence interval = 0.59–1.97, $P = 0.8$). Similarly, the 3161G variant was no more common in cases than in controls (2.9% versus 2.2%, $P = 0.2$), there was no difference in genotype distribution between cases and controls ($P = 0.1$), and the CG genotype was not associated with an increased risk of breast cancer (adjusted odds ratio = 1.30, 95% confidence interval = 0.85–1.98, $P = 0.2$). This lack of evidence for an association persisted within groups defined by the family history of breast cancer or by age.

Conclusion: The 2119C and 3161G amino acid substitution variants are not associated with moderate or high risks of breast cancer in Australian women.

Keywords: ataxia-telangiectasia mutated gene, breast cancer, variant

Introduction

The major known familial breast cancer predisposition genes *BRCA1* and *BRCA2* are involved in DNA repair [1]. The ataxia-telangiectasia mutated (*ATM*) gene product functions in the double-strand break repair pathway [2], and there is evidence to suggest that some *ATM* gene

ATM = ataxia-telangiectasia mutated gene; CI = confidence interval; OR = odds ratio; PCR = polymerase chain reaction.

mutations increase the risk of breast cancer. Studies of families with ataxia-telangiectasia linked to the *ATM* region report an average fourfold elevated frequency of breast cancer among female obligate heterozygote *ATM* gene mutation carriers [3]. In addition, *ATM* mutations with dominant-negative effects have been detected in a proportion of Australian multiple-case breast cancer families [4].

It has been suggested that some *ATM* missense variants may be more common in breast cancer cases selected for first-degree family history and early age at onset [5]. Furthermore, the T2119C Ser707Pro variant [5–7] and the β-adaptin domain C3161G Pro1054Arg variant [8] have been reported to be breast cancer related. These findings were based on small studies of less than 150 cases or controls, with the exception of one large study of 1000 cases and 500 controls [6], and the confidence intervals of risk estimates are wide. We have undertaken a study to investigate the breast cancer risk associated with these two nonconservative amino acid substitution variants using a large Australian population-based case–control study of more than 1300 cases and 600 controls, more than one-half of whom were aged younger than 40 years.

Materials and methods

Subjects

The Australian Breast Cancer Family Study, a population-based, age-stratified, case–control–family study of first primary invasive breast cancer in women younger than age 40 years was carried out in Melbourne and Sydney from 1992 to 1995 [9–11]. The study was extended from 1996 to 2000 to also include women up to age 59 years [10]. Cases were women with a diagnosis of a first primary breast cancer identified through the Victorian and New South Wales cancer registries. Controls were women without breast cancer selected from the electoral roll (adult registration for voting is compulsory in Australia) using stratified random sampling, frequency-matched for age.

Cases and controls were administered a questionnaire to record the family history of cancer and other known or potential risk factors for breast cancer. With the subjects' permission, all living parents, aunts, grandparents, and adult siblings were asked to participate, and were administered the same risk-factor questionnaire [10,11]. Ancestry was assessed by an open-ended question, and from the country of birth of the respondents, their parents and their grandparents. The great majority of the subjects' parents and grandparents were born in Australia, the British Isles or Western Europe. In subanalyses restricted to Caucasian women, subjects either with any Australian aboriginal, Torres Strait Islander or Maori heritage or with any country of birth in the South Pacific, Indian Ocean, Caribbean islands or Asia were excluded.

The family history of cancers was systematically collected from each case and each control, and included the cancer history of all of their first-degree and second-degree relatives. This history was subsequently checked with each living relative at the time of their interview. A family history of breast cancer was defined as having at least one first-degree or second-degree relative with breast cancer. Verification of all cancers reported by subjects and their relatives was sought through personal interview, cancer registries, pathology reports, hospital records, clinicians and death certificates.

Subjects participating in the study conducted from 1996 to 2000 were asked for information on the number of X-ray examinations or radiation treatments they had undergone, and their age at first X-ray examination or radiation treatment. Exposure to X-rays/ionizing radiation was considered as a yes/no variable, irrespective of age at first exposure.

Interviews were conducted for 1579 of 2304 eligible cases (68.5%) and for 1021 of 1531 eligible controls (66.7%). Attrition of cases was due to death (1.8%), to refusal by the surgeon (8.5%), to refusal by the proband (16.4%), to nonresponse by the surgeon (1.3%), to nonresponse by the proband (1.2%) and to failure to locate the proband (2.3%). Attrition of controls was due to refusal (28.2%) and to nonresponse (5.1%). Not all interviewed cases and controls elected to donate a blood sample for DNA studies. Genotyping for the T2119C variant was carried out on the 1331 cases (84% of those participating) and 649 controls (64% of those participating) with DNA available at the time of analysis, and subsequently for the C3161G variant on 1453 cases (92% of those participating) and 793 controls (78% of those participating). PCR success rates were greater than 99%. The average age ± standard deviation of cases and controls was 39.6 ± 9.0 and 42.0 ± 8.8 years, respectively, for individuals genotyped for the T2119C variant, and was 41.7 ± 8.7 and 40.1 ± 8.9 years, respectively, for individuals genotyped for the C3161G variant.

Approval of this study was obtained from the ethics committees of The University of Melbourne, the New South Wales Cancer Council, The Anti-Cancer Council of Victoria, and The Queensland Institute of Medical Research.

Molecular analysis

Collection of peripheral blood and DNA extraction have been described previously [12]. The *ATM* variants were detected using the ABI Prism 7700 Sequence Detection System 5′ exonuclease assay (Perkin-Elmer Corp., Foster City, USA), using the methodology as described previously [13]. The T2119C variant was detected using the forward and reverse primers 5′-CGCTGTCTTCTGGGTTTATCAG-3′ and 5′-CCTTCCTAACAGTTTACCAAAGTTGA-3′, and the probes 5′-FAM-CTGAATAATTACTCAt-

CTGAGGTGAGAT-TAMRA-3′ (T allele) and 5′-VIC-TCTGAATAATTACTCAcCTGAGGTGA-TAMRA-3′ (C allele). The C3161G variant was detected using the forward and reverse primers 5′-CTCTATTTCATATTTAACCACAGTTCTTTTC-3′ and 5′-GTCTTTTCCCATTACATTAAGAATGG-3′, and the probes designed to the complement strand 5′-FAM-CCCATTTTGAATAAgGATCAGCCTACGG-TAMRA-3′ (C allele) and 5′-VIC-CCCATTTTGAATAAcGATCAGCCTACGG-TAMRA-3′ (G allele).

Statistical methods

Allele frequencies were estimated and compared assuming that alleles within an individual were independent binomial variables. The Hardy–Weinberg equilibrium assumption was assessed for defined groups using maximum likelihood methods by comparing the observed numbers of different genotypes with those expected under Hardy–Weinberg equilibrium in that group. The odds ratio (OR) and the 95% confidence interval (CI) were calculated using unconditional logistical regression, with and without adjustment for measured risk factors. We used stratified analysis to determine whether the genotype-breast cancer association varied by age <40 or ≥ 40 years, by family history, by menopausal status and by chest exposure to X-rays and/or ionizing radiation. All statistical tests and *P* values were two tailed and, following convention, statistical significance was taken as a nominal $P<0.05$. SPSS (version 10.0; SPSS Australia Pty Ltd), Epi-Info 6 (freeware; http://www.cdc.gov/epiinfo/ei6.htm) and Ottutil software (freeware; http://linkage.rockefeller.edu/ott/linkutil.htm) were used for the statistical analyses.

Results

Table 1 presents the *ATM* T2119C and C3161G allele and genotype frequencies for cases and controls overall, and stratified by family history of breast cancer (reported first-degree or second-degree relative) or by age (<40 years versus ≥ 40 years). There was no evidence of deviation from Hardy–Weinberg equilibrium for either variant, in either cases or controls ($P \geq 0.3$).

The 2119C variant was rare, occurring at frequencies of 1.4 and 1.3% in cases and controls, respectively ($P=0.8$). There was no difference in genotype distribution between cases and controls ($P=0.8$), and the TC genotype was not associated with an increased risk of breast cancer (adjusted OR = 1.08, 95% CI = 0.59–1.97, $P=0.8$). Similarly, the 3161G variant was no more common in cases than in controls (2.9% versus 2.2%, $P=0.2$), there was no difference in genotype distribution between cases and controls ($P=0.1$), and the CG genotype was not associated with an increased risk of breast cancer (adjusted OR = 1.30, 95% CI = 0.85–1.98, $P=0.2$).

This lack of evidence for an association persisted within groups defined by family history of breast cancer or age (Table 1) ($P>0.2$), for both family history and age (data not shown) ($P>0.2$), or for menopausal status (post-menopausal status defined as cessation of menstrual periods for 1 year or more; data not shown) ($P>0.6$). Crude risk estimates were similar to those adjusted for measured potential confounders (Table 1). ORs were also little different when adjusted only for the subset of factors that might influence risk estimates due to the possibility of distorted control selection (namely, age, country of birth, state, education and marital status), in which case the ORs became 1.16 (95% CI = 0.64–2.09) and 1.33 (95% CI = 0.88–2.01) for the 2119 TC and 3161 CG genotypes, respectively.

Results were similar when analyses were restricted to women of Caucasian ancestry. The adjusted OR was 1.14 (95% CI = 0.60–2.14) for the 1185 cases and 573 controls genotyped for the T2119C variant ($P=0.7$), and was 1.37 (95% CI = 0.87–2.17) for the 1296 cases and 660 controls genotyped for the C3161G variant ($P=0.2$). The ORs were also little different when analyses were carried out excluding cases known to carry a deleterious mutation in *BRCA1* or *BRCA2* (32 and 34 individuals for T2119C and C3161G, respectively; data not shown).

Since *ATM* homozygote and heterozygote mutation carriers are known to exhibit radiosensitivity, and radiation exposure is associated with risk of breast cancer, we studied the T2119C and C3161G genotyped cases (244 and 263 individuals, respectively) and controls (87 and 105 individuals, respectively) who reported exposure to chest X-rays and/or ionizing radiation. Although the frequency of the variant genotype was numerically greater in exposed cases compared with exposed controls for both variants, this difference was not significant for either T2119C (2.5% versus 1.1%, OR = 2.17, 95% CI = 0.26–18.27, $P=0.5$) or C3161G (3.8% versus 2.9%, OR = 1.34, 95% CI = 0.36–4.98, $P=0.7$).

Finally, we investigated the possibility that the risk of breast cancer may be modified by a combination of the two variants. There were no individuals who were heterozygous for both variants and, compared with the 2119 TT/ 3161 CC reference group, there was no increased risk of breast cancer associated with having the 2119 TC genotype alone (OR = 1.11, 95% CI = 0.62–1.98, $P=0.7$), the 3161 GC genotype alone (OR = 1.36, 95% CI = 0.88–2.13, $P=0.2$), or either variant (i.e. the 2119 TC genotype or the 3161 CG genotype) (OR = 1.27, 95% CI = 0.89–1.81, $P=0.2$).

Discussion

The 2119C variant has been reported by three studies to be more common in breast cancer cases than in controls [5–7]. Using subsamples from a population-based, case–control study carried out in three different

Table 1

Breast cancer risk and the ataxia-telangiectasia mutated gene (*ATM*) T2119C and C3161G variants

	Family history[a]		No family history		< 40 years		≥ 40 years		Total		Crude OR		Adjusted OR	
Genotype	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases[b]	Controls	(95% CI)	*P*	(95% CI)[c]	*P*
ATM T2119C														
TT	428 (97.5)	158 (98.1)	859 (97.0)	473 (97.1)	677 (97.0)	421 (97.2)	610 (97.3)	210 (97.7)	1287 (97.1)	631 (97.4)	Reference		Reference	
TC	11 (2.5)	3 (1.9)	27 (3.0)	14 (2.9)	21 (3.0)	12 (2.8)	17 (2.7)	5 (2.3)	38 (2.9)	17 (2.6)	1.10 (0.61–1.96)	0.8	1.08 (0.59–1.97)	0.8
Total	439	161	886	487	698	433	627	215	1325	648				
C allele frequency	0.013	0.009	0.015	0.014	0.015	0.014	0.014	0.012	0.014	0.013				
95% CI	0.005–0.020	0.000–0.020	0.010–0.021	0.007–0.022	0.009–0.021	0.006–0.022	0.007–0.020	0.001–0.022	0.010–0.019	0.007–0.019				
ATM C3161G														
CC	439 (93.6)	175 (95.1)	922 (94.6)	579 (95.9)	722 (94.4)	422 (95.9)	639 (94.1)	332 (95.4)	1361 (94.3)	754 (95.7)	Reference		Reference	
CG	30 (6.4)	9 (4.9)	53 (5.4)	25 (4.1)	43 (5.6)	18 (4.1)	40 (5.9)	16 (4.6)	83 (5.7)	34 (4.3)	1.35 (0.90–2.03)	0.1	1.30 (0.85–1.98)	0.2
Total	469	184	975	604	765	440	679	348	1444	788				
G allele frequency	0.032	0.024	0.027	0.021	0.028	0.020	0.029	0.023	0.029	0.022				
95% CI	0.021–0.043	0.009–0.040	0.020–0.034	0.013–0.029	0.020–0.036	0.011–0.030	0.020–0.038	0.012–0.034	0.023–0.035	0.014–0.029				

Data presented as *n* (%). OR, odds ratio; CI, confidence interval.

[a] Family history defined as any reported first-degree or second-degree relative with breast cancer. The 3161 heterozygote genotype was detected in 1/49 (2%) of the subgroup of cases reporting affected sibs.

[b] To date, 32 and 34 cases included in the T2119C and C3161G analysis, respectively, have been found to have a deleterious mutation in *BRCA1* or *BRCA2* by protein-truncation testing in specific exons covering about 70% of the coding regions, and by manual sequencing of *BRCA1* in a subset.

[c] Adjusted ORs were adjusted for age, country of birth, state, education, marital status, number of live births, height, weight, age at menarche, oral contraceptive use, and for reported family history of breast cancer (first-degree or second-degree relative).

geographic areas of the United States, Teraoka *et al*. [5] observed the 2119C variant in 6/142 (4.2%) cases selected for diagnosis before the age of 35 years, or diagnosis before the age of 45 years *and* reporting a first-degree family history of breast cancer, and in 1/81 (1.2%) controls of similar age. This translates to an OR (95% CI) of 3.5 (0.4–29.9). A Georgetown study of 43 breast cancer patients and 43 ethnically matched controls, sampled at a medical centre and a clinic, respectively, reported the 2119C variant in a single case only (2.3%) [7]. A large study of individuals from the Lower Saxony region of Germany reported heterozygote frequencies of 2.8% in 1000 unselected hospital-based breast cancer patients (median age, 57 years) and of 1.2% in 500 random blood donor controls, translating to an OR of 2.4 (95% CI = 1.0–5.6) that was at best marginally different from unity [6]. Our larger Australian population-based case and control samples were frequency-matched for age, and our study found no evidence for such an increased risk, before or after adjustment for measured risk factors, or in the subset of Caucasian individuals. Our results also suggest that the risk associated with the 2119C allele is unlikely to be limited to younger women reporting a family history, as reported by Teraoka *et al*. [5].

The previous study reporting breast cancer risk associated with the 3161G variant used both a case–control and sibpair design [8]. The heterozygote genotype was observed in 2/57 (3.5%) sporadic breast cancer cases, in 4/126 (3.2%) controls, and in 9/66 (13.6%) affected cases identified from sibpairs with breast cancer. The OR within the latter group was reported to be 4.5 (95% CI = 1.2–20.5). The present study found no evidence for such an effect. Although the frequency of the 3161 heterozygote genotype is nominally greater in Australian cases compared with controls, overall or within strata defined by family history or age, this difference is not statistically significant. Furthermore, the frequency of heterozygotes in our subgroup of 49 cases reporting affected sibs (2.0%) was decreased relative to controls, and was not statistically significantly different ($P = 0.06$) to the sibship cases of Larson *et al*. [8]. Interestingly, the Georgetown study of 43 breast cancer patients and 43 ethnically matched controls [7] also found no evidence to suggest that the 3161G variant is associated with breast cancer, reporting the 3161G variant at similar frequencies among cases (two heterozygotes) and controls (one heterozygote and one homozygote).

Given the observed frequencies of greater than 1% for the variant alleles in our large control sample ($n > 600$), and no large differences in allele frequency between the case and control groups, we believe these variants should be considered nonpathogenic polymorphisms. These variants are unlikely to be associated with even moderate risks of breast cancer, although we cannot exclude the possibility that they may be associated with weaker risks.

The present study was of sufficient size to have 80% power at the 0.05 level of significance to detect an OR of 2.1 or more for the T2119C variant and an OR of 1.8 or more for the C3161G variant. Even if the observed OR point estimates of 1.1 and 1.3 are true, however, the rarity of the variants is such that the population-attributable risks associated with them would be in the order of only 0.3–1%. We also cannot exclude a modest increased risk in women exposed to radiation, despite having genotyped more than 1900 women. Evaluations of gene–environment interaction require much larger sample sizes, particularly given the rarity of exposure to high-level radiation, and the likelihood of substantial misclassification of both high-level and low-level radiation exposure due to retrospective exposure assessment. Large cohort studies of radiation-exposed women would be needed to assess the association of *ATM* gene variants with breast cancer risk in such women, but the value of such studies from a population health perspective is questionable given the rarity of both genotype and exposure.

Conclusion

We found no evidence that the *ATM* 2119C and 3161G amino acid substitution variants are associated with moderate or high risks of breast cancer in Australian women. Although weak risks cannot be excluded, our results suggest that the population-attributable risk associated with them would be negligible.

Acknowledgements

The authors are grateful to physicians, surgeons and oncologists in Victoria, New South Wales and Queensland who endorsed this project, to the interviewing staff, and to the many women and their relatives who participated in this research. This work was supported by the Queensland Cancer Council, the Susan Komen Foundation (USA), the National Health and Medical Research Council of Australia, the Victorian Health Promotion Foundation, the NSW Cancer Council, the Peter MacCallum Cancer Institute, the Inkster-Ross Memorial Fund, and the National Institutes of Health, as part of the Cancer Family Registry for Breast Cancer Study (CA 69638). The authors thank Sarah Ellis-Steinborner, Melissa Southey and Deon Venter for preparation of DNA, and thank Gillian Dite for the risk factor information. The authors are indebted to Thilo Dörk and Francine Durocher for *ATM* T2119C and C3161G positive controls.

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