Award Number: DAMD17-99-1-9260

TITLE: The Importance of ATM Mutations and Polymorphisms in Breast Cancer and Radiation Sensitivity

PRINCIPAL INVESTIGATOR: Thomas A. Buchholz, M.D.

CONTRACTING ORGANIZATION: The University of Texas M.D. Anderson Cancer Center
Houston, Texas 77030

REPORT DATE: October 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
**REPORT DOCUMENTATION PAGE**

<table>
<thead>
<tr>
<th>1. AGENCY USE ONLY (Leave blank)</th>
<th>2. REPORT DATE</th>
<th>3. REPORT TYPE AND DATES COVERED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October 2001</td>
<td>Annual Summary (1 Oct 00 – 30 Sep 01)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. TITLE AND SUBTITLE</th>
<th>5. FUNDING NUMBERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Importance of ATM Mutations and Polymorphisms in Breast Cancer and Radiation Sensitivity</td>
<td>DAMD17-99-1-9260</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6. AUTHOR(S)</th>
<th>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas A. Buchholz, M.D.</td>
<td>The University of Texas M.D. Anderson Cancer Center Houston, Texas 77030</td>
</tr>
<tr>
<td></td>
<td>E-Mail: <a href="mailto:tbuchhol@mdanderson.org">tbuchhol@mdanderson.org</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8. PERFORMING ORGANIZATION REPORT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</th>
<th>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>11. SUPPLEMENTARY NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12a. DISTRIBUTION / AVAILABILITY STATEMENT</th>
<th>12b. DISTRIBUTION CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approved for Public Release; Distribution Unlimited</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13. ABSTRACT (Maximum 200 Words)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The objective of my 4-year Career Development Award was to determine whether ATM heterozygosity contributes to breast cancer development and radiation injury. We sequenced the ATM cDNA of 93 breast cancer patients, 22 of whom experienced a normal tissue injury from radiation treatment. We found that none of these patients had an ATM mutation that resulted in a protein truncation. This finding is consistent with publications from others. We did identify 3 repetitive single-base changes in the ATM cDNA that may represent missense mutations. We then compared the frequency of these single-base changes between the breast cancer patients and a control set of samples from 996 individuals without cancer. We found that one of these single-base changes was more commonly represented in the breast cancer patients (6.7% vs 1.6%, p=0.006). To further assess whether this base change results in a functional consequence, we developed an in vitro assay to study the role the ATM protein plays in repair of double-strand DNA damage. We now plan to study cells with the identified single-base changes using this assay. We hypothesize that these single base changes affect a cellular phenotype previously shown to have relevance to breast cancer development and radiation injury risk.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>14. SUBJECT TERMS</th>
<th>15. NUMBER OF PAGES</th>
<th>16. PRICE CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>17. SECURITY CLASSIFICATION OF REPORT</th>
<th>18. SECURITY CLASSIFICATION OF THIS PAGE</th>
<th>19. SECURITY CLASSIFICATION OF ABSTRACT</th>
<th>20. LIMITATION OF ABSTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassified</td>
<td>Unclassified</td>
<td>Unclassified</td>
<td>Unlimited</td>
</tr>
</tbody>
</table>

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89) Prescribed by ANSI Std. Z39-18 298-102
Table of Contents

Cover.........................................................................................................................1
SF 298.....................................................................................................................2
Table of Contents......................................................................................................3
Introduction...............................................................................................................4
Body..........................................................................................................................5-8
Key Research Accomplishments.............................................................................9
Reportable Outcomes..............................................................................................10-12
Conclusions..............................................................................................................13
References...............................................................................................................14
Appendices..............................................................................................................15-18
Introduction

The focus of this research program is to investigate the relationship between abnormalities in the ATM gene and the susceptibility for breast cancer and normal tissue injury following breast cancer radiation treatment. Our original plan was to sequence ATM cDNA in 200 breast cancer patients and 50 breast cancer patients with a significant radiation injury over the 4-year award period. Our rationale for studying ATM in breast cancer and radiation sensitivity arose after obligate ATM heterozygotes (family members of children with ataxia telangiectasia) were noted to have a 5-fold greater risk of developing breast cancer than the general population (1). This lead to estimates that 8% of all breast cancers occur in ATM heterozygotes (2). For our initial studies, we enrolled 111 breast cancer patients onto our ATM protocol. The sequencing of ATM failed in 18 patients leaving 93 for analysis. We did not find a protein truncating mutation in any of the first 93 breast cancer patients in whom we sequenced the ATM cDNA. Instead, we found that 37 of the 93 patients had at least one single-base substitutions in the gene. Three of the single-base substitutions were found in more than one patient. The lack of protein truncating mutations and the presence of single-base substitutions in the gene have also recently been reported by a number of other investigators (3-5). Single-base substitutions in the gene can represent polymorphisms that have no functional consequence or may represent missense mutations that change the protein product and affect the cellular phenotype. To investigate the significance of our finding, we changed the focus of our research from continuing cDNA sequencing in additional breast cancer patients to instead focusing on testing whether these single-base gene changes were clinically relevant to breast cancer development. We developed two strategies to determine the clinical relevance of the identified single-base changes. First, we began testing whether the single-base change was over-represented in breast cancer patients compared to individuals without a cancer history. Our second strategy has been to develop an in vitro assay to determine whether the single-base changes affect protein complexes involved in double-strand DNA damage repair. Based on our preliminary data in this line of research, we plan to focus our future efforts over the next two years specifically in this area of investigation.
Body

The first specific aim in our statement of work was to establish the incidence of ATM heterozygosity in 200 breast cancer patients. This aim was to be completed over the first 3 years of the funding period. We reported many of the details of the sequencing work in the first annual report of this award and will again highlight and update these efforts. We had developed a cDNA sequencing process for the ATM gene prior to the time of awarding of this grant. The sequencing process was verified by confirming the presence of a mutation in two obligate ATM heterozygotes (parents of an individual with the disease of ataxia telangiectasia). During the first two years of the funding period, we enrolled 111 breast cancer patients onto our institutional protocols studying the ATM gene. As specified in this task, the coding of all clinical and epidemiological information was abstracted and recorded for all of these patients. From these samples, RNA was isolated and transcribed into cDNA and then sequenced for mutations in ATM. Sufficient information from sequencing of ATM cDNA was obtained in 93 unrelated breast cancer patients.

Our first specific aim focused on studying a nonselected breast cancer patient population. No protein truncating mutations were found in the 93 breast cancer who had ATM cDNA sequenced. However, a total of 37 patients had variation of the sequenced gene compared to the sequence listed in GenBank. Specifically, a total of 41 single base changes were detected in the 37 patients. Nine patients had 2 or more single nucleotide changes. From these patients, we identified 4 missense mutations/polymorphisms that occurred repeatedly in more than one patient. The specific polymorphism and their frequency are listed in the below:

- Ser49Cys
  This change was seen in 5/75 patients (the denominator does not total the total patient number because the entire gene sequence was not obtained in every patient sample).

- Asp1853Asn
  This change was seen in 19/58 patients.

– Ser707Pro
  This change was seen in 4/76 patients.

– Pro1054Arg
  This change was seen in 3/61 patients.

Our second specific aim in our statement of work was to establish the incidence of ATM heterozygosity in 50 breast cancer patients experiencing a significant acute or late normal tissue radiation injury over the first 3 years of the funding period. In the first year of this award, we have sequenced the cDNA of 22 breast cancer patients who had radiation complications. No protein truncating mutations were found in this cohort. A total of 6 patients had variation of the genes compared to that listed in GenBank. Specifically, a total of 8 single base changes were detected in the 6 patients. Two patients had 2 single nucleotide changes. The frequency of these polymorphisms / missense mutations were:

1. Ser49Cys (shown above): 1/17
2. Asp1853Asn (shown above): 5/16
3. Ser707Pro (shown above): 0/16
4. Pro1054Arg (shown above): 2/19

The final specific aim of the research project was to compare the rates of mutations in our case population and controls. A significant effort of our research in the second year of this funding has been developing an allele specific oligonucleotide assay that could be used for high-throughput screening of a
large population for the presence or absence of a specific polymorphism. The development of this assay proved to be more difficult than anticipated, but over the past year we have been successful in developing such an assay for three of the above polymorphisms: Ser49Cys, Asp1853Asn, and Pro1054Arg. Using the ASO assay we began testing our 996 control population for the presence of these single-base changes. As polymorphisms in genes can have ethnic and sex variations, we first tested whether the frequency of these base changes differed amongst different races and sexes and found the following:

Ser49Cys – no ethnic or gender variations
Asp1853Asn – varied by both ethnicity and gender
Pro1054Arg – varied by ethnicity but not gender

Based on the following we analyzed the entire population frequency for the Ser49Cys change, only Caucasian women cases and controls for Asp1853Asn, and only Caucasian cases and control for the Pro1054Arg. There were no homozygous mutations for Ser49Cys and Pro1054Arg so we reported our results as frequency of having the base-change per individual. There were homozygous mutations for the Asp1853Asn and so we reported allelic frequency.

The table below summarizes our results:

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Cases</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser49Cys</td>
<td>6.7%</td>
<td>1.6%</td>
<td>P=0.006</td>
</tr>
<tr>
<td>Asp1853Asn</td>
<td>18.1%</td>
<td>14.4%</td>
<td>P=0.246</td>
</tr>
<tr>
<td>Pro1054Arg</td>
<td>4.9%</td>
<td>6.8%</td>
<td>P=0.587</td>
</tr>
</tbody>
</table>

We interpreted these data as suggesting that the Ser49Cys single-base change may be relevant to breast cancer formation whereas the Asp1853Asn and the Pro1054Arg likely represent polymorphisms. We further studied the clinical and epidemiological characteristics of the cases with the Ser49Cys and found that this base change was even more common in women with bilateral breast cancer. Specifically, the rate of the base change in 17 bilateral breast cancer patients was 11.8%, which was significantly higher than the control group rate, P=0.025. The rate of the base change was not increased in the 17 patients with a radiation complication in whom the Ser49Cys region was evaluated.

These data suggest that the Ser49Cys may represent a missense mutation that has relevance to breast cancer development. These data represent a novel finding that has significant relevance to breast cancer research. An abstract highlighting these findings was accepted for presentation at this year’s ECCO11 – the European Cancer Conference meeting that is to be held this month in Lisbon, Portugal. The acceptance letter is included as an attachment with this renewal. In addition, we are currently in the process of completing a manuscript to report this finding.

This is an important finding that will need to be confirmed in an independent data set. Towards that end, we propose studying the frequency of the polymorphism in an independent set of 20 bilateral breast cancer patients. We will use the ASO assay to test for the Ser49Cys missense mutation.

In addition to the specific tasks outlined below, we have been developing a functional assay to study ATM. We felt that such an assay would enable us to not only show that the Ser49Cys base-change is more common in breast cancer patients, but also show that it leads to functional and molecular consequences. Towards this end, we have developed an electrophoretic mobility shift assay (EMSA) and over the past year have accumulated sufficient preliminary data to suggest that this assay can be used to identify ATM-containing molecular complexes involved in the repair of DNA double-strand breaks. This is a relevant focus of study because a number of other tumor suppressor gene mutations, such as BRCA1 and BRCA2, may predispose an individual to breast cancer development by affecting the cellular repair of double-strand DNA damage. In fact, it has been recently reported that ATM phosphorylates BRCA and it is possible that
dysfunction in the ATM protein may then affect an individual’s risk via the same pathway as germline mutation in BRCA1 or BRCA2 (6,7).

Based on this rationale, I developed a new collaboration with a colleague in my department, Craig Stevens, M.D., Ph.D. who was using the EMSA assay to study DNA-PK containing molecular complexes in Chinese Hamster Cells (8). We began using this technique to study molecular complexes involved in double-strand break repair in human skin fibroblast samples. We identified that humans have distinctly different EMSA band patterns compared to rodents. An example of the banding patterns seen in humans and rodents are shown in Figure 1 below. We then obtained human cells with homozygous ATM mutations and found that they lacked 6 EMSA bands (also shown in Figure 1). These data suggested that the ATM protein was important for the formation of these DNA end-binding molecular complexes. To test whether the ATM protein was a part of these complexes, we performed a supershift assay, in which an ATM antibody is added to the complex and it is determined whether the antibody leads to a shift in the molecular weight of the band of interest. The results of this study showed the ATM is present in these molecular complexes (figure 2). We are currently in the process of completing a manuscript to report these findings.

Figure 1. Comparison of normal mouse and human EMSA banding patterns. There are at least 10 EMSA bands when EMSA is done with human nuclear extract. AT cells which lack a functional ATM protein, are missing six EMSA bands compare with normal human controls.

Figure 2. Anti-ATM antibodies alter the mobility of all of the bands (arrows at left) which are absent in AT cells.
Our current plan is to study whether the ATM-containing molecular complexes that bind to double-strand breaks are affected by the ATM single-base changes. We will be studying the single-base changes that others and we have discovered. A finding that polymorphisms decrease the level of these complexes would add further support of them representing a missense mutation. This represents a new focus to our research that we did not anticipate at the time of our original representation. However, we think this avenue of research has significant clinical relevance to determining a relationship of the ATM gene and breast cancer development. A number of investigators are now reporting data that single-base changes may be associated with breast cancer formation and we are not aware of another group investigating EMSA banding patterns to understand the molecular etiology of this association.

A final focus of this new avenue of research will be to investigate whether cells with known single-base changes affect ATM kinase activity. To study this, we will study ATM kinase activity in fibroblast nuclear extracts by assessing phosphorylation of PHAS-1 and Nbs-1 with and without rapamycin.

In addition to my research investigating the importance of the ATM gene and breast cancer development, the two years of funding from the Career Development Award has had a significant impact on the development of my career as a physician/scientist. With support from this grant, I have been able to entirely focus my career on the treatment and research of breast cancer. The support from this award over the past two-years has also allowed me to conduct and publish a number of clinical research studies and present research results in a number of national/international conferences. I have listed the publications in which the support from the Career Development Award was acknowledged in the manuscript. In addition, my national/international breast cancer presentations during the period of my award are also listed.
Key research accomplishments (Years 1 and 2)

- Sequenced ATM cDNA from 93 breast cancer patients
- Identified 4 repetitive single nucleotide base changes resulting in an amino acid change in the protein
- Established a control bank of DNA from 996 individuals without a cancer history for population comparison studies
- Developed an allele specific oligonucleotide assay for 3 repetitive single nucleotide base changes in the ATM gene
- Established a collaborative effort that demonstrated the feasibility of using haplotype association studies to detect single nucleotide changes in the ATM gene.
- Demonstrated the bilateral breast cancer patients have an increased number of radiation-induced chromatid breaks compare to control showing the feasibility of a phenotype rather than genotype assay to predict breast cancer risk.
- Identified that the Ser49Cys single-base change is over-represented in breast cancer patients compared to controls
- Identified that the Ser49Cys single-base change is over-represented in patients with bilateral breast cancer compared to controls
- Established a collaborative effort that identified the EMSA binding pattern of human cells.
- Established a collaborative effort that identified six specific EMSA bands that were lost in human cells with homozygous mutations.
- Demonstrated that the ATM protein is present in these six EMSA molecular complexes
- Developed the EMSA band pattern as an assay to test the functional significance of identified ATM polymorphisms
Reportable Outcomes

- manuscripts, abstracts, presentations:

**Published Articles In Which the Support from the Career Development Award Was Acknowledged**


**Journal Articles In Press In Which the Support from the Career Development Award Was Acknowledged**


**Invited Editorials During Period of Career Development Award – (Award was not cited).**


- patents and licenses applied for and/or issued: none
- degrees obtained that are supported by this award: none
- development of cell lines, tissue or serum repositories
1. established a control bank of DNA from 996 individuals with no cancer history to serve for population frequency testing

- informatics such as databases and animal models, etc:

  1. established a database for enrolled patients and controls that cover patient demographics, cancer history, and toxicity from radiation treatment.

- funding applied for based on work supported by this award: none

- employment or research opportunities applied for and/or received on experiences/training supported by this award: none.
Conclusion

Over the first 2 years of this 4-year program, we have been successful in sequencing ATM cDNA in breast cancer patients and breast cancer patients with radiation injury. Based on these studies, we conclude that these populations have multiple single nucleotide base changes that may have contributed to their disease and treatment-related toxicity. To explore this relationship further, we successfully developed allele specific oligonucleotide assays and identified a single nucleotide change that is more common in breast cancer patients compared to controls.

In addition to the continuing the planned studies outlined in our original statement of work and body of this report, we hope to begin assaying whether some of the single base changes result in cellular radiosensitivity and deficiency in DNA damage repair. We have opened and institutional review board approved protocol to obtain skin fibroblasts from individuals in whom our sequencing studies have identified single nucleotide changes. The fibroblasts will be grown in culture for in vitro assays of radiosensitivity, DNA damage repair, and biochemistry assays.

Establishing a relationship between ATM and breast cancer development and/or normal tissue toxicity following breast cancer radiation treatment would be a significant contribution to breast cancer research. If our data identifies an association between specific single nucleotide changes and breast cancer, further studies will be needed to determine the penetrance rate. Ultimately, allele specific oligonucleotide assays or an assessment of EMSA band patterns could serve as a simple tool to screen large populations of women to further define their breast cancer risk and risk of treatment-related toxicity.
References


Dr. T. Buchholz  
M.D. Anderson Cancer Center  
Radiation Oncology  
1515 Holcombe Blvd.  
Houston, Texas 77030  
USA  

June 26, 2001  

Dear Dr. Buchholz  

I am pleased to inform you that your abstract "Over-representation of a polymorphism/misense mutation in the ataxia telangiectasia, mutated (ATM) gene in breast cancer patients versus controls" has been selected by the Scientific Committee for presentation in a poster discussion session during ECCO 11.  

Please find attached an overview of the poster discussion session on "Breast cancer biology" which is scheduled on Wednesday, 24 October 2001 from 13.00 to 13.45 and will be held in Hall 4. You are invited to summarize the highlights of your work in a 5 minutes oral presentation. In addition, your poster will not be displayed in the large group of posters but on a poster board in the lecture hall itself. Please make sure to mount your poster in Hall 4, either before 8.30 a.m. on the day of the presentation or, at the latest, between 12.00 and 12.30 on that same day. The poster will be displayed on the day of the presentation.  

For the short presentation, a maximum of 5 slides is allowed (only slides - no powerpoint presentation). You should ensure that your slides are brought to the slide preview room, the evening before your presentation or before 10.00 am on the day of the presentation. Slides should be collected immediately after the session.  

The new number for your abstract in the programme and abstract book is 971. This is also your number on the poster board.  

Please note that the acceptance of your abstract does not provide you with a free registration to the conference and that you should register at your earliest convenience. If for any reason you would not be able to attend ECCO 11, please arrange for one of the co-authors of your abstract to give the presentation. Please notify the ECCO 11 secretariat, as early as possible, of such a replacement.  

For your information, please note that it is of utmost importance to arrange for your travel and stay in Lisbon as soon as possible because flights and hotel rooms are being booked up fairly quickly for ECCO. Top Tours, the official accommodation agent for ECCO 11 is pleased to assist you but you are free to arrange for accommodation with any other travel agent. Top Tours contact details are: e-mail: congress@toptours.pt tel. 351-21-3169800; fax 351-21-3525285.  

Congratulations on the acceptance of your abstract and I look forward to meeting you in Lisbon.  

Yours sincerely  

[Signature]  

Professor Harald zur Hausen  
Chairman Scientific Committee (Basic Science/Medics)
<table>
<thead>
<tr>
<th>Start</th>
<th>Function</th>
<th>Name</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00</td>
<td>Speaker</td>
<td>Ingvarsson, S.</td>
<td>Mutation analysis of the CHK2 gene in breast carcinoma and other cancers.</td>
</tr>
<tr>
<td>13:18</td>
<td>Speaker</td>
<td>Gee, Julia</td>
<td>The EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') is an effective inhibitor of tamoxifen-resistant breast cancer growth</td>
</tr>
<tr>
<td>13:09</td>
<td>Speaker</td>
<td>Mass, R.D.</td>
<td>Fluorescence in situ hybridization (FISH) may accurately identify patients who obtain survival benefit from herceptin plus chemotherapy</td>
</tr>
<tr>
<td>13:27</td>
<td>Speaker</td>
<td>Buchholz, Thomas</td>
<td>Over-representation of a polymorphism/missense mutation in the ataxia telangiectasia, mutated (ATM) gene in breast cancer patients versus controls</td>
</tr>
<tr>
<td>13:36</td>
<td>Speaker</td>
<td>Schindlbeck, Chr</td>
<td>Comparison of the prognostic significance of occult metastatic cells in the bone marrow (OMC-BM) and HER2-status in patients with stage I-III breast cancer (BC)</td>
</tr>
</tbody>
</table>
Over-representation of a Polymorphism/Missense Mutation in the Ataxia Telangiectasia, Mutated (ATM) Gene in Breast Cancer Patients Versus Controls

Buchholz TA, Story MD, Ashorn CL, Chakraborty R, Strom EA, Cox JD, McNeese MD, and Weil MM

Purpose: Mothers of children diagnosed with ataxia telangiectasia have been reported to be at increased risk for breast cancer development. To test whether germline mutations in the ATM gene are associated with breast cancer, we compared the frequency of ATM cDNA sequence changes in breast cancer patients and controls.

Methods: We sequenced ATM cDNA in 91 breast cancer patients and compared sequence changes in these patients to the frequency of these alterations in a control set of 996 individuals with no cancer history. An allele specific oligonucleotide assay was used to study the specific polymorphisms of interest in the ATM cDNA for the control set. The frequency of identified base changes was also tested across ethnic groups and gender.

Results: No mutations that would lead to protein truncation were identified, but several polymorphisms were found in the cDNA of the breast cancer patients. The three polymorphisms that were found in two or more patients cause amino acid substitutions in the ATM protein of the following type: Ser49Cys, Pro1054Arg, and Asp1853Asn. The Ser49Cys polymorphism was found in 6.7% (5/75) of the breast cancer patients.
compared to 1.6% (12/946) of the control group (P=0.006, Fisher's 2-sided exact). The subgroup of patients with bilateral breast cancer had a frequency rate of 11.8% (2/17) which again was significantly different from the control group (P=0.025, Fisher's 2-sided exact). None of the 9 breast cancer patients that had a normal tissue complication following radiation treatment had the Ser49Cys change. The allelic frequencies of the other two polymorphisms were not different between cases and controls.

**Conclusion:** Breast cancer patients, particularly those with bilateral disease, are more likely to have a polymorphism in the ATM gene that results in a Ser49Cys change in the protein compared to controls. These data suggest Ser49Cys may be a functional polymorphism that contributes to breast cancer development or a polymorphism that is linked to another causative genetic factor.