Advances in Brief

The Role of Ataxia-Telangiectasia Heterozygotes in Familial Breast Cancer

Jindong Chen, Gabriella Giesler Birkholtz, Per Lindblom, Carlos Rubio, and Annika Lindblom

Clinical Genetics Unit, Department of Molecular Medicine, Karolinska Institute [J. C. G. G., P. L. A. L.], and Department of Pathology, Karolinska Hospital [C. R.], S-17176 Stockholm, Sweden

Abstract

The role of ataxia-telangiectasia (AT) heterozygotes in breast cancer has been controversial. We have found previously an overrepresentation (3.4%) of ATM mutations in a subset of 88 selected breast cancer patients with a family history of breast cancer, leukemia, and lymphomas. This prevalence is comparable to the estimated value (3.8%) from epidemiological study. To further examine the possibility that ATM is correlated to breast cancer, we screened for ATM germ-line mutations in another 100 breast cancer patients with a family history of breast cancer. We used the protein truncating test and found one new germ-line mutation. This figure (1%) is consistent with the observed 0.2–1% carrier frequency for AT. We also studied breast tumors from ATM mutants, and three showed retention of both alleles, whereas the fourth showed loss of the mutant allele. We conclude that the contribution of heterozygous ATM mutations to familial breast cancer is minimal. Even if the ATM gene were causative in these cases, it is not likely to act as a tumor suppressor.

Introduction

AT3 is an autosomal recessive disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, immune defects, and a predisposition to malignancy. The gene for AT, designated ATM, located on chromosome 11q22-q23, contains 66 exons spanning approximately 150 kb of genomic DNA and encodes a protein of 3056 amino acids (1–3). It is well established that patients with AT have a strong predisposition to malignancy, particularly leukemias and lymphomas (4, 5). An increased predisposition to malignancy has also been suggested to involve heterozygotes for ATM gene mutations (6–10).

The increased risk for heterozygotes was most prominent for breast cancer (6, 7), with an estimated risk of 5.1 over that in the general population (7), and a high proportion (3.8%) of breast cancer cases may be carriers of AT mutations (8). To date, a few studies including our first study have been conducted to verify this hypothesis (2, 11–14). However, these publications presented discrepant results. The study conducted by Athma et al. (14) reported a significantly increased risk of breast cancer in female mutation carriers in AT families. We have also found previously an overrepresentation (3.4%) of ATM mutations in a subset of 88 selected breast cancer patients with a family history of breast cancer, leukemia, and lymphomas (13). However, in the studies by FitzGerald et al. (11), Wooster et al. (12), and Cortessis et al. (15), no support for an increased risk was found.

Clearly, the issue of AT heterozygosity and breast cancer is highly significant and needs to be clarified. To further examine the possibility that ATM is associated with breast cancer, we selected 100 additional breast cancer patients with a family history of breast cancer for screening of ATM mutations. We have in previous studies character-ized the mutation spectrum in these families by screening for germ-line mutations in the TP53, the BRCA1, and the BRCA2 genes. The lack of germ-line mutations in TP53 (16) and the low mutation incidence identified in BRCA1 (17) and BRCA2 (18) in these families indicated that other genes, including ATM, are likely to play a role in the occurrence of familial breast cancer. The observation that chain-terminating mutations account for 90% of mutations identified in children with AT (19–23) made us select the PTT to screen for germ-line mutations. One patient from each family was selected for analysis.

Patients and Methods

Collection of Patients. One hundred breast cancer families (not including the 88 families in the previous study) with a family history of breast cancer were collected at the Cancer Family Clinic in Karolinska Hospital during 1990 to 1995. These families were selected with a criteria of two or more cases of breast cancer, regardless of age of onset. In total, we studied one affected woman from each family. The families showed different numbers of breast cancer cases: 2 families with six breast cancer cases, 7 families with five breast cancer cases, 12 families with four cases, 36 families with three cases, and 43 families with only two cases of breast cancer. We had previously excluded germ-line mutations in the BRCA1 (14) and the BRCA2 (18) genes in these patients. Thus, because these families do not segregate the high penetrant breast cancer genes BRCA1 or BRCA2, the majority present with a low number of breast cancer cases, and because they are selected regardless of age of onset, they represent families with a high likelihood of segregating low penetrant breast cancer genes, such as ATM.

DNA and RNA Preparation. DNA was isolated from whole blood, and lymphocytes were isolated by standard phenol/chloroform extraction. Total RNA was extracted from EBV-transformed lymphocytes by acid guanidinium thiocyanate-phenol-chloroform extraction. All tumors were obtained from paraffin-fixed materials and cut in 5-μm-thick sections. Normal and tumor tissue was identified, and DNA was extracted using a conventional method.

RT-PCR. Uncoding exons 1a, 1b, 2, and the 3′-untranslated region contained in exon 65 were not examined. The entire coding sequence of ATM transcript, composed of 63 exons covering 9.2 kb, was divided into nine overlapping fragments for PTT analysis (Table 1), and RT-PCR was needed to produce transcription templates for PTT. All of the sequences of primers are given in Table 1. Each forward primer carried a T7 promoter and an eukaryotic translation initiation sequence. Total RNA (2–5 μg) was reverse transcribed with random hexamers using the superscript cDNA preamplification kit (Life Technologies, Inc.) to generate cDNA. A 2-μl aliquot of cDNA was used for subsequent PCR. PCR reactions were carried out in a volume of 50 μl containing 50 ng of genomic DNA, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM each of dATP, dGTP, dCTP, and dTTP, each primer at 0.2 μM, and 2 units of Taq (Perkin-Elmer) or DynaZyme (Finnzymes) polymerase. Amplification was run for 45 cycles of 40 s at 94°C, 50 s at 53–57°C, 1 min 30 s at 72°C, and a final extension step for 10 min at 72°C.

PTT Analysis. A 13-μl reaction mixture containing 5 μl of RT-PCR product, 6.25 μl of TnT rabbit reticulocyte lysate (Promega), 0.5 μl of [35S]methionine (1000 Ci/mmol, NEN Life Science), 0.25 μl TnT T7 RNA polymerase, 0.25 μl of amino acid methionine(-), and 0.25 μl of RNAAsin (recombinant, 40 units/μl) was incubated at 30°C for 1.5 h. Following the PTT, 5 μl of product was electrophoresed on a 15% SDS-polyacrylamide gel. Following the electrophoresis, the gel was dried and subjected to autoradiography overnight.
Table 1 PTT primers used for mutation screening of the ATM gene

<table>
<thead>
<tr>
<th>Name of primer</th>
<th>Region</th>
<th>Sense[5'→3']</th>
<th>Antisense[5'→3']</th>
<th>Annealing temp.</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT-3/10</td>
<td>23-1684</td>
<td>ATGGTTATCTGGTGGCCGTC</td>
<td>TTTGCTCTATTTCCCATTTTACC</td>
<td>53°C</td>
<td>1734</td>
</tr>
<tr>
<td>PTT-11/15</td>
<td>992-2260</td>
<td>AGTATTCTCTAGAAGTTTGCG</td>
<td>CATGAGAGGATGTCCTTCT</td>
<td>57°C</td>
<td>1305</td>
</tr>
<tr>
<td>PTT-16/22</td>
<td>2009-3203</td>
<td>GTATTAGAAAAACCCCATCCTCA</td>
<td>AGGTCTTCCCATATTCAATTT</td>
<td>55°C</td>
<td>1231</td>
</tr>
<tr>
<td>PTT-20/28</td>
<td>2732-4141</td>
<td>CTCTGAGACCATACCTGC</td>
<td>ATGGAGGTTATTAGGAGCG</td>
<td>53°C</td>
<td>1446</td>
</tr>
<tr>
<td>PTT-28/35</td>
<td>3797-5170</td>
<td>ATGGAGGAGTACCCATCG</td>
<td>CTACCCAGTGTATTACCCAG</td>
<td>54°C</td>
<td>1410</td>
</tr>
<tr>
<td>PTT-35/46</td>
<td>4919-6695</td>
<td>AAGATGGGAATTATGTTGAA</td>
<td>TCCAAATGACTCGTCGTAAG</td>
<td>56°C</td>
<td>1813</td>
</tr>
<tr>
<td>PTT-45/50</td>
<td>6221-7355</td>
<td>GCCATATCTCTTGGTCTTA</td>
<td>AGCGCTATTCATCCACTATC</td>
<td>55°C</td>
<td>1171</td>
</tr>
<tr>
<td>PTT-50/59</td>
<td>6974-8616</td>
<td>CGACAGATCCCAGGCTAAA</td>
<td>AGTGTCTATCCAGTGTCAAA</td>
<td>53°C</td>
<td>1679</td>
</tr>
<tr>
<td>PTT-55/65</td>
<td>7919-9181</td>
<td>CTGACAGAAAAGCCATATT</td>
<td>ATAGTCTAGAATCGCACCACCA</td>
<td>57°C</td>
<td>1299</td>
</tr>
</tbody>
</table>

* A T7 promoter sequence and translation start codon were added to all of the sense primers at their 5' end. The T7 promoter sequence with a translation start site is 5'-GCTAATACGACTCACTATAGGAGACAGACCATG-3'.

** Temp., temperature.

Direct DNA Sequencing. Any PCR fragment producing a truncated protein, along with the wild-type product, was subjected to cycling sequencing (Amersham Life Science). Two μl of PCR product were used for sequencing. The sequencing reaction was carried out in a volume of 7 μl containing 2–2.5 pg of DNA, 2 pm sequencing primer, 0.5 unit polymerase (Amersham Life Science), 0.5 μl of [3P]dNTP (Amersham Life Science), and 2 μl of dGTP mixture. The samples were denatured at 95°C for 3 min, followed by 45 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. After amplification, 3 μl of stop solution (95% formamide, 10 mM NaOH, and 0.25% bromphenol blue) were added. The mixture was denatured at 70°C for 2 min, and 3.5 μl of the mixture were electrophoresed on a 6% sequencing gel in 1x TBE buffer (0.090 M Tris-borate, 0.002 M EDTA). Gels were fixed, dried, and exposed to film at room temperature for 12 h or more.

Results and Discussion

To date, the work of Swift et al. (6, 7) has been controversial. Verifying Swift's finding has been difficult because AT carriers have no clinical phenotype and lack distinguishing in vitro cellular characteristics. By using linkage analysis in breast cancer families, Wooster et al. (12) and Cortessis et al. (15) first tested the hypothesis that AT heterozygotes suffer an increased risk of breast cancer. They found no evidence for linkage between familial breast cancer and their markers and concluded that the contribution of AT to familial breast cancer is likely to be minimal. But the likelihood methods used in these studies lacked sufficient power to exclude the possible relationship between AT heterozygotes and breast cancer for such a complex human trait. Following the isolation of the ATM gene in 1995 (1–3, 24), Vorechovsky et al. (2) screened for AT heterozygotes in 38 sporadic breast cancer patients by single-strand conformational polymorphism, and no mutations were identified. In our first study of 88 patients from breast cancer families with lymphoma/leukemia and gastric cancer cases, three AT heterozygotes were identified (13). This observation (3.4%) was higher than the expected carrier frequency (0.2–1%), comparable with the estimated 3.8%. This result is inclined to support the epidemiological finding that AT heterozygotes have an increased risk for breast cancer (6, 7, 25) and might suggest that AT heterozygosity is associated with susceptibility to breast cancer, as observed previously (6–9). Recently, however, two studies on the relation between AT heterozygotes and breast cancer have been undertaken from two opposite directions, irrespective of family history of breast cancer (11, 14), and discrepant results have been obtained. One of them conducted by Athma et al. (14), by following identified AT mutations through the families of those with clinically recognized AT, found evidence of an increased risk, similar to that found by Easton (25). The other study, carried out by FitzGerald et al. (11), however, by screening the general population of breast cancer patients for unknown ATM mutations, tended to be against the work of Swift et al. (6, 7). In the publication of FitzGerald et al. (11), ATM mutations were detected in 2 of 401 (0.5%) women diagnosed with early onset of breast cancer and in 2 of 202 (1%) controls, consistent with the frequency of AT carriers predicted from epidemiological studies.
AT HETEROZYGOTES IN FAMILIAL BREAST CANCER

In this study, we identified one new germ-line mutation in 100 breast cancer families. This is a Glu2990ter nonsense mutation (8968G>T; Fig. 1), leading to a loss of the last 66 amino acids. It was identified in a woman from a family (family 4043) with three breast cancer cases and was inherited from her mother, who also had breast cancer. The index case was diagnosed with breast cancer at 35 years of age. The observed prevalence (1%) is consistent with the AT carrier frequency range (0.2-1%) in Caucasians predicted from epidemiological studies (25, 26). The accurate incidence of AT carriers in the Swedish population is not known. It was roughly estimated to be consistent with the lower range (0.2-0.5%) of the above estimate (13). Our data may be slightly underestimated because PTT was designed to identify truncating mutations. That means we might have missed some uncommon missense mutation(s) or in-frame deletion(s) of <25 bp (27) or possible mutations in ATM gene regulatory regions. Of the mutations identified in ATM thus far, however, more than 90% result in a premature termination of translation (19; Human Gene Mutation Database).

To further establish whether the germ-line mutation in this family was causative, we decided to study the tumors for loss of heterozygosity. Because the homozygotes for AT mutations have a very high risk for malignancy, the AT gene is hypothesized to function as a tumor suppressor gene in tumorigenesis. In this case, one would expect the wild-type allele to be lost in the tumors. The tumors from the patient and her mother showed retention for both the mutated and the wild-type alleles (Fig. 2C). It seems that ATM was unlikely to act as a tumor suppressor gene in this breast cancer family.

Then we reexamined the three germ-line mutations, which were identified in the previous study of 88 breast cancer families (13). Both constitutional and tumor DNA were analyzed. Two tumors (AL18-FBC11 and AL7-FBC33 from families 3024 and 621, respectively), also displayed retention of both the mutated and the wild-type alleles in the tumors (Fig. 2B and C), whereas the third one (482-89DFBC42 from family 498) showed loss of the mutated allele (Fig. 2D). This suggests that not even in these families was the AT gene the predisposing factor. Our data constitute the first molecular evidence that the ATM gene is not causative in familial breast cancer, or at least not acting as a tumor suppressor gene based on Knudson’s two-hit hypothesis.

Fig. 2. The DNA sequencing of both blood and tumors from the patients with ATM mutations. A–C, the retention of both normal and mutated ATM alleles in the tumors from patient Br60 and her mother (M-, patient’s mother; P-, patient Br60). AL18-FBC11 (3245 delATC insTGAT) and AL7-FBC33 (7636 del9), respectively; arrowhead in C, start of deletion. D, loss of mutated ATM allele in the tumor from the patient 482-89DFBC42 with mutation 2114 insT (exon 14); arrowhead, inserted T. Nor, normal; Mut, mutated sequence. The PCR primers have been described by Vorechovsky et al. (2, 13). The reverse primer was used as sequencing primer in this study.
Acknowledgments

We are indebted to the family members for their cooperation, Anne-Lise Børresen-Dale for providing us with control materials, and Bert Vogelstein for valuable suggestions.

References

The Role of Ataxia-Telangiectasia Heterozygotes in Familial Breast Cancer

Jindong Chen, Gabriella Giesler Birkholtz, Per Lindblom, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/7/1376

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.