ATM Mutations in Cancer Families

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Abstract

Ataxia-telangiectasia (A-T) is a multisystem recessive disease characterized clinically by cerebellar ataxia, oculocutaneous telangiectasias, immunodeficiency, sensitivity to radiomimetic agents, and cancer predisposition. This pleiotropic disorder is caused by mutations in the ATM (mutated in A-T) gene, which is located in the human chromosomal region 11q22–q23. The ATM gene product is a member of a novel family of large proteins implicated in the regulation of the cell cycle and response to DNA damage. Heterozygosity for A-T was previously suggested to be associated with an increased risk of tumors, particularly female breast cancer. Because a loss of constitutional heterozygosity at 11q22–q23 is a frequent event in breast and other tumors, suggesting the presence of a tumor suppressor gene(s) in this region, we screened blood DNA samples from 88 unrelated breast cancer patients of Swedish cancer families for ATM mutations using single-strand conformation polymorphism analysis. All patients had a family history of tumors previously associated with A-T heterozygosity or homozygosity. We demonstrate the first three germ-line mutations in ATM identified by screening of breast cancer patients. Two mutations were previously found in A-T homozygotes and one mutation was a 1-bp insertion. All mutations were found in families with a large number of tumors, however, they did not cosegregate with malignancies. Although the proportion of A-T carriers in this sample seems to be higher than expected by chance, larger studies and pooled data sets will be required to establish that an A-T allele confers cancer susceptibility in heterozygotes.

Introduction

A-T is a multisystem recessive disorder characterized by cerebellar degeneration with progressive ataxia, oculocutaneous telangiectasias, immunodeficiency, sensitivity to radiomimetic agents, and cancer predisposition (for a review, see Ref. 1). The disease locus was mapped to the chromosomal region 11q22–q23 using genetic linkage analysis (2). The gene for A-T, designated ATM (mutated in A-T), was identified by positional cloning (3, 4) and found to be widely expressed as a 13-kb transcript with minor species of various sizes (3). The deduced amino acid sequence of 3056 residues revealed similarities to the catalytic domain of phosphatidylinositol-3'-kinases at the carboxy-terminal part (3), Drosophila melanogaster mei41, Saccharomyces cerevisiae TOR1 and TOR2 (and their mammalian homologues FRAP and rRAFT), MEC1, Schizosaccharomyces pombe rad3, and a DNA-dependent protein kinase catalytic subunit, forming a new family of ATM-related genes. This gene family is implicated in cell cycle regulation, control of telomere length, and/or response to DNA damage (5).

The risk of cancer in patients with A-T is about 100 times higher than in the non-A-T population (6). A-T homozygotes carrying two copies of a mutated ATM gene often develop lymphoid malignancies in childhood, and they also seem to be at risk of epithelial tumors such as gastric cancer (7). Individuals heterozygous for A-T have also been suggested to have a higher incidence of tumors (8, 9). The risk of cancer in A-T heterozygotes was found to be particularly high for breast carcinomas in females (8, 9). The pooled analysis by Easton, based on previously published studies and an updated United Kingdom study (8–11), estimated the relative risk of breast cancer to A-T heterozygotes at 3.9 (12). Because A-T heterozygotes constitute about 0.2–1% of the general population (12), a significant fraction of breast carcinomas may develop in carriers of an A-T allele. This proportion was estimated at 3.8%, assuming a disease gene frequency of 0.005 and a relative risk of 3.9 (12).

Recently, the genomic organization of the complete ATM gene was determined (13–15). We have developed an exon-scanning PCR-SSCP assay for detection of unknown mutations in ATM (14). We did not detect any somatic mutations in ATM in breast tumor DNA, indicating that this gene is not a frequently altered tumor suppressor gene in sporadic breast cancer (14). We were unable to demonstrate any germ-line A-T alleles among 38 unselected breast tumor patients (14). Here we have analyzed blood DNA of 88 unrelated index cases with primary breast cancer. Each index case had a family history of breast cancer and/or a family history of tumors previously associated with A-T heterozygosity or homozygosity. If A-T alleles confer cancer susceptibility in heterozygotes, one would expect an enrichment for A-T carriers in such families as compared to the general population.

Materials and Methods

Patients. Eighty-eight unrelated female patients with familial cancer were included in the mutation study of ATM. They were ascertained through the Karolinska Hospital’s Clinic for Familial Cancer. The selection criteria for including the patients in the study involved: (a) the occurrence of primary breast cancer in the index case; and (b) the presence of at least one additional case of breast cancer, lymphoma, leukemia, or gastric cancer among close family members. Sixty-eight index cases had familial breast cancer. In addition, 19 index cases had a close family relative who developed leukemia; 16 of them were from multiple-case breast cancer families. Seven index cases had a family history of lymphoma, and six of them also had a family history of breast cancer. The average age at diagnosis of index cases was 53 (±12) years (±SD), ranging from 24–79 years (median, 52 years). Ninety-six to 126 chromosomes were analyzed in control females for those segments exhibiting rare or unique SSCP alterations.

Blood Samples and DNA Extraction. Blood samples taken from the index cases and their family members were stored frozen until analysis. DNA was extracted using methods as described (14).

PCR-SSCP Analysis. Altogether, 65 segments of the ATM gene covering about 9.2 kb of the coding sequence and 6.5 kb of the flanking introns were

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3 The abbreviations used are: A-T, ataxia-telangiectasia; ATM, gene mutated in A-T; SSCP, single-strand conformation polymorphism.

4130
amplified individually from patients' DNA. The oligonucleotide primer pairs amplifying all ATM coding exons were described previously, together with PCR conditions for each pair (14). The SSCP analysis was performed as described (14, 16). The segments containing band shifts were cut from the gel (17), re-amplified, and sequenced directly using a Sequenase PCR Product sequencing kit (Amersham, Little Chalfont, United Kingdom).

Statistical Analysis. The allelic frequencies observed in breast cancer patients and those estimated in the general population were compared using the nomenclature for mutations/polymorphisms proposed by Beaudet and Tsui (34) is used.

Results and Discussion

ATM Polymorphisms. SSCP analysis of 65 PCR segments individually amplified from 88 DNA samples showed an altered SSCP pattern in a total of 13 PCR segments. These included polymorphic sites in segments corresponding to exons 3, 11, 25, 38, and 62. These common polymorphisms, which had similar allelic frequencies in breast cancer patients and controls, were described previously (14). In addition, eight rare two-allelic polymorphisms were found in this series of individuals (Table 1). These polymorphisms, comprising both intronic and exonic changes, had a frequency of rare alleles close to the frequency of the disease allele, but, with the exception of three alterations (Table 1), all rare alleles were also found in controls. The amino acid substitution in exon 35 affects a nonconserved residue, the deletion in intron 58 would not be predicted to interfere with mRNA splicing, and the silent polymorphism in exon 14 does not create a splice site (Table 1). Because most A-T alleles contain null mutations in ATM (18, 19), we consider these changes rare polymorphisms. No rare alleles were identified in segments corresponding to exons 15, 31, 44, 48, and 61 in this series of patients.

A-T Carriers in Cancer Families. In addition to a number of polymorphic sites in the gene, three ATM mutations were found in exons 14, 23, and 53 (Fig. 1 and Table 2). They were all found in patients from families with a high number of tumors (Fig. 2, a–c). The heterozygous mutation in exon 14 was previously identified in a Swedish patient with A-T by analyzing 76 chromosomes in patients with familial cancer and 126 chromosomes in control females except for exons 18 and 59.

of ATM is unclear. The second mutation, a 9-bp in-frame deletion in exon 53, was also observed previously in A-T patients. It was found in two homozygotes and one compound heterozygote of Irish origin (18) and in two more compound heterozygotes in the United Kingdom study (19). The number of tumors in this cancer family was also high, including three cases of gastric cancer (Fig. 2c). The third mutation detected in exon 23 was a 1-bp heterozygous insertion (Table 2; Figs. 2b and 2b).

Although the accurate frequency of A-T heterozygotes in the Swedish population has not been estimated previously, the frequency of the disease allele in Caucasians was suggested to be 0.2–1.0%, with 0.5% being the best estimate (12). Only five cases were, to our knowledge, diagnosed in Sweden over the last 10 years, which would be consistent with the lower range of the above estimate. Assuming the population prevalence of the disease allele of 0.2–0.5%, one would expect 0.18–0.44 A-T carriers among 88 individuals with breast cancer. The three A-T carriers identified in our families (3.4%) seem to be a higher number than expected by chance (P = 0.086, odds ratio 7.0 and 0 = 0.012, odds ratio 17.6, assuming a population frequency of the disease allele of 0.005 and 0.002, respectively). The frequency of breast cancer patients carrying an A-T allele was comparable to that estimated previously (12) and might suggest that A-T heterozygosity is associated with susceptibility to cancer as observed previously (8–11).

However, in the absence of data on the disease allele frequency in the studied population, these results should be considered preliminary. A-T heterozygotes might not have been ascertained completely, leading to an underestimation of the frequency of A-T carriers in cancer families.

Table 1. Summary of rare polymorphisms in patients with familial cancer

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change&lt;sup&gt;a&lt;/sup&gt; and protein&lt;sup&gt;b&lt;/sup&gt; change</th>
<th>Allelic counts&lt;sup&gt;c&lt;/sup&gt; in patients with familial cancer</th>
<th>Allelic counts&lt;sup&gt;c&lt;/sup&gt; in the general population</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>145C→G</td>
<td>G: 1</td>
<td>G: 2</td>
</tr>
<tr>
<td>14</td>
<td>1985T→C</td>
<td>C: 1</td>
<td>C: 0</td>
</tr>
<tr>
<td>23</td>
<td>3160C→G</td>
<td>C: 1</td>
<td>C: 0</td>
</tr>
<tr>
<td>27</td>
<td>3992 + 40G→A</td>
<td>A: 4</td>
<td>A: 1</td>
</tr>
<tr>
<td>30</td>
<td>4257C→T</td>
<td>G: 1</td>
<td>G: 125</td>
</tr>
<tr>
<td>35</td>
<td>5070A→C</td>
<td>C: 1</td>
<td>C: 0</td>
</tr>
<tr>
<td>59</td>
<td>8368.54deTAGTTT</td>
<td>Del: 1</td>
<td>Del: 0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The nucleotide position corresponds to the sequence given in Ref. 4. 
<sup>b</sup> The first methionine in the open reading frame is at amino acid position +1. 
<sup>c</sup> Allelic counts were determined from SSCP analysis of 65 PCR segments individually amplified from 88 DNA samples. 

Table 2. A-T heterozygotes in cancer families

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exon</th>
<th>Nucleotide change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Predicted protein change&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL18-FBC11</td>
<td>23</td>
<td>3246insG</td>
<td>Frameshift</td>
</tr>
<tr>
<td>AL7-FBC33</td>
<td>14</td>
<td>7636del9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>DelS2546, R2547, and 12548</td>
</tr>
</tbody>
</table>

<sup>a</sup> The nucleotide position corresponds to the sequence given in Ref. 4. 
<sup>b</sup> The first methionine in the open reading frame is at position +1. 
<sup>c</sup> The deleted sequence was 5'-CTAGAATTT-3'.

Fig. 1. The SSCP pattern of three heterozygous ATM mutations. A, patient AL7-FBC33 with deletion in exon 53; B, patient AL18-FBC11 with mutation in exon 23; C, patient 482–89DFBC42 with exon 14 mutation. Lanes exhibiting band shifts are surrounded by two controls on each side.
Fig. 2. Pedigrees of three cancer families with index cases carrying an A-T allele. a, the exon 14 mutation found in patient 482–89DFBC42 (Table 2) and her son; b, the exon 23 mutation found in patient AL-18-FBC11 (Table 2) and her brother and two nieces; c, the exon 33 mutation found in patient AL7-FBC33 (Table 2). The age at diagnosis of malignancy is shown below the symbol, if known. M, mutated ATM allele; N, normal genotype; NA, not analyzed.

The A-T phenotype may be more common because there might be individuals carrying subtle mutations in the ATM gene not necessarily resulting in the major clinical features of A-T but still interfering with cellular functions of ATM. This hypothesis would be supported by the low consanguinity rate observed in A-T families (20) and the exist-

Symbol definitions

- Unaffected
- Stomach cancer
- Breast cancer
- Leukemia
- Lung cancer
- Head and neck cancer
- Prostate cancer
- Melanoma
- Myeloma
- Renal carcinoma
- Gynecol. cancer
- Hepatic cancer
ence of variant forms of A-T. To determine the disease allele frequency using molecular analysis would require screening thousands of individuals carefully selected to avoid the effect of population stratification, a task beyond the means of many laboratories. However, much larger studies and pooled analyses will be required to establish that the risk of cancer is indeed conferred by A-T alleles and to accurately assess the significance of mutated ATM alleles in cancer susceptibility.

A-T Alleles Do Not Co-segregate with Tumors in Cancer Families. To study co-segregation of mutations with the malignant phenotype, SSCP analysis was carried out in the available pedigree members in parallel with the index cases. The mutation in exon 14 was found in the offspring of the proband with breast cancer (Fig. 2a). The son of this A-T carrier inherited the mutated ATM allele from his mother and developed melanoma at the age of 47. The mutation in exon 23 was absent in proband’s brother, who developed myeloma (Fig. 2b). One unaffected sister and two daughters, who are currently free of breast cancer and are ages 63 and 46, are carriers of the mutation (Fig. 2b). In patient AL7-FBC33 (Fig. 2c), the samples from other pedigree members were not available for analysis.

As expected, the complex tumor phenotype did not co-segregate with A-T alleles in these families. However, these results do not exclude ATM as a breast cancer susceptibility gene. Although genetic linkage analyses in multiple-case families failed to provide evidence of the linkage of breast cancer to 11q22–q23 (21, 22), the likelihood methods used in these studies lacked sufficient exclusion power for such a complex human trait.

Clearly, larger linkage studies are needed using affected pedigree members and methods less vulnerable to phenocopies, incomplete penetrance, incorrect segregation models, genetic heterogeneity, and multiple environmental effects (23, 24). The presence of mutations in the offspring of affected A-T heterozygotes warrants a prospective follow-up and risk assessment of newly identified carriers.

Numerous studies have observed a frequent loss of constitutional heterozygosity at 11q22–q23 in breast (25–30) and other (31, 32) cancers and methods less vulnerable to phenocopies, incomplete penetrance, and ascertained pedigrees revealed no evidence of linkage. Cancer Res., 55: 2726–2732, 1995.


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