Spectrum of ATM Gene Mutations in a Hospital-based Series of Unselected Breast Cancer Patients

Thilo Dörk, Regina Bendix, Michael Bremer, Dirk Rades, Karin Klöpper, Marion Nicke, Britta Skawran, Ariadne Hector, Paria Yamini, Diana Steinnmann, Sabine Weise, Manfred Stührmann, and Johann H. Karstens

Department of Biochemistry and Tumour Biology, Clinic of Obstetrics and Gynecology [D. R., M. N., J. H. K.], Institute of Human Genetics [M. N., B. S., M. S.], and Department of Haematology and Oncology [D. S.], Medical School Hannover, D-30659 Hannover; and Center for Child Neurology, Hospital Gerresheim, D-40225 Düsseldorf [S. W. J., Germany]

ABSTRACT

Blood relatives of patients with the inherited disease ataxia telangiectasia (A-T) have an increased susceptibility for breast cancer. We therefore looked for sequence alterations of the ATM gene in a large hospital-based series of unselected breast cancer patients. The whole ATM coding sequence was analyzed in genomic DNA samples from a core group of 192 consecutive breast cancer cases to define the spectrum of ATM gene mutations. Common sequence alterations were then screened in the whole series of 1000 breast cancer patients and in 500 random individuals. In the core group, 21 distinct sequence alterations were identified throughout the ATM coding region, and 1 common splicing mutation was uncovered in intron 10. Almost half of the breast cancer patients (46%) were heterozygotes for 1 of 16 different amino acid substitutions, and three patients (1.6%) carried a truncating mutation. These data indicate that ~1 in 50 German breast cancer patients is heterozygous for an A-T-causing mutation. In our extended series, the most common A-T mutation 1066-6T→G was disclosed in 7 of 1000 (0.7%) breast cancer patients. Transcript analyses indicated that the loss of exon 11 in the ATM mRNA was the pathogenic consequence of this splicing mutation, which produced a <10% of full-length ATM mRNA and ATM protein in a homozygous A-T patient. We also found an excess of rare missense substitutions in the breast cancer cohort compared with random individuals (7.9% versus 5.3% of alleles; odds ratio = 1.6; P < 0.01). One missense substitution, S707P in exon 15, was twice more frequent in breast cancer patients (odds ratio = 2.4; 95% confidence interval, 1.0–5.8) and five times more frequent in patients with bilateral disease than in random individuals (P < 0.001). We conclude that a large variety of distinct ATM mutations and variants exist among breast cancer patients, some of which may contribute to the etiology and progression of the malignancy. Screening for frequent A-T mutations such as the 1066-6→G splice site substitution can be effective to prospectively identify A-T heterozygotes in an unselected cancer patient population.

INTRODUCTION

Several risk factors for breast cancer have been defined, including age, family history, hormonal factors, and radiation exposure (1–3). Apart from the two familiar breast cancer genes BRCA1 and BRCA2, it is thought that mutations in genes with lower penetrance may explain much of the hereditary predisposition to breast cancer (3). One candidate is ATM, the gene mutated in A-T (4, 5). The ATM gene consists of 66 exons encoding a large protein kinase that orchestrates the recognition and repair of radiation-induced DNA double strand breaks (5–9). Several oncoproteins are regulated by ATM, including the tumor suppressors p53 and BRCA1 (6, 10). A-T patients have a high incidence of cancer (11–13), and some adult patients with A-T have been reported to develop breast cancer (13, 14).

Life expectancy is reduced in A-T heterozygotes, who may account for ~1% of the general population, because of an increased appearance of age-related disorders (15). Several epidemiological studies have provided strong evidence for an increased frequency of malignancies, particularly breast cancer, among blood relatives of patients with A-T (11, 16–23). A-T heterozygotes appear to have a risk of breast carcinoma that is ~3.8 times greater than that of noncarriers, leading to the estimation that carriers of an ATM gene mutation may account for 6.6% of all breast cancer cases (20). Direct molecular examination of selected breast cancer cohorts outside of A-T families has led to conflicting, albeit not mutually exclusive, results (3, 24–33). In a recent report, Broeks et al. (32) detected seven germline ATM mutations among 82 breast cancer cases who had either early-onset disease or bilateral breast cancer; the authors concluded that truncating germline mutations of ATM contribute to breast cancer susceptibility. By contrast, FitzGerald et al. (26) detected ATM mutations in only 2 of 401 (0.5%) breast cancer patients with onset <40 years and concluded that truncating mutations of ATM do not predispose to early-onset breast cancer. Within A-T families, the relative risk of breast cancer appears to be highest, i.e., 6–7-fold increased, in obligate A-T heterozygotes 50–69 years of age (20), a range similar to the median age at onset of breast cancer in the general population. We initiated a population-based study to define the spectrum and elucidate the clinical relevance of ATM gene mutations in a large series of unselected breast cancer patients treated at the same hospital.

PATIENTS AND METHODS

Patients. Peripheral EDTA blood samples were collected, after written informed consent had been obtained, from 1000 consecutive breast cancer patients who received postoperative radiotherapy at the Medical School Hannover from September 1995 to April 1999. All patients were residents of Lower Saxony, a region in the north of Germany. Median age at onset of breast cancer was 57 years in this patient cohort (range, 27–85 years). Of these patients, 26.9% had developed breast cancer below the age of 50 years and 7% below the age of 40 years; 6.7% had bilateral breast cancer. The involved breast was irradiated postoperatively with a 6 MV photon beam of a linear accelerator with a mean total dose of 52 Gy (range, 45–54 Gy; single dose, 1.8 Gy), followed by a boost with electrons (10–14 Gy) to the tumor bed in 6.8% of the patients. The ipsilateral peripheral lymph nodes were irradiated in 14% of patients (total dose 45 Gy, single dose 1.8 Gy). Only 1 of the 1000 patients showed severe acute toxicity, scored as grade III according to common toxicity criteria (34), and 2 developed severe late reactions related to local radiotherapy, which were classified as grade III and IV, respectively, according to the LENT-SOMA score criteria (3b), a relatively low proportion which may be related to the sequential application of chemotherapy and radiotherapy. Several of the patients (28%) reported at least one blood relative with breast cancer. Fourteen patients had been identified as carriers of a frequent BRCA1 or BRCA2 gene mutation in a parallel study (data not shown); these patients were left within the cohort to keep the study group unbiased and to take into account the possibility of double heterozygosity. Genomic DNA was extracted from leukocytes of all patients and served as the primary source for mutational screening. For comparison, a series of 500 genomic DNA samples were
collected from random blood donors from the same geographic region who had been anonymized to keep confidentiality. These samples served merely to confirm a detection rate of 75% of A-T mutations with this exon-scanning approach (data not shown). PCR products with an aberrant migration on the SSCP gel were sequenced on both strands using the BigDye Terminator Cycle Sequencing Kit and an ABI 310 sequencer (Perkin-Elmer) to identify the underlying sequence alteration. Using mutation-specific restriction enzyme-based screening assays, we subsequently screened for frequent mutations and variants in the total cohort of 1000 breast cancer patients and in 500 anonymous blood donors from the general population of Lower Saxony. We used AlwII to test for the presence of the P1054R substitution, MboII to test for the presence of the L1420F substitution, and Rsul to test for the presence of the 1066-G mutagenesis. The S707P substitution was screened using the primers 5'-TAAGGCGAAGCATTTAGTCATG-3' and 5'-TTTCTCTCTTCTCTCAGTTTACC-3' followed by RsuI digestion. A specific mismatch primer (5'-GGAGGATCAGTCATCCATGAATGTA-3') was used in combination with the reverse primer (5'-GGGAAACCTCAAAAGGCTATAC-3') to screen for the F858L substitution by RsuI digestion. Similarly, splicing mutation 3576G->A was screened using mismatch primer 5'-GGATTAGAACCTGCACCTCCTCGGAAA-3' together with the reverse primer 5'-CCTAGTCTTAAATAAGTGCCACTC-3', followed by digestion with EcoNI. The substitutions D1853N and D1853V were screened for and distinguished by SSCP analyses of exon 39 PCR products.

We investigated the effect of mutation 1066-6T->G on splicing, using RNA samples obtained from the lymphoblastoid cell lines of a homozygous A-T patient (HA141) and of unrelated individuals who did not carry this mutation, as well as from peripheral blood lymphocytes of three breast cancer patients heterozygous for the mutation. Total RNA was reverse-transcribed using random hexamer primers and a First-strand-cDNA synthesis Kit (Amersham/Pharmacia). One-fifth of the cDNA served as the template for a subsequent PCR using primers 5'-GATCTGCTAGTGAATGAGATAAGTC-3' and 5'-CATGGAAGCTGCAGCTGACCCA-3', with the forward primer being fluorescence labeled (annealing at 57°C; 30 cycles). The labeled PCR products, 659 bp for product containing exon 11 and 489 bp for product lacking exon 11, were separated on a denaturing 5% polyacrylamide gel on an A.L.F. sequencer (Amersham/Pharmacia), and their relative quantities were determined by Fragment Manager 1.2 software (Amersham/Pharmacia). Values given for the homozygous patient are the mean of three measurements from either of two different RNA preparations. Expression of ATM protein was determined by Western blot analyses of lymphoblastoid cell extracts from the 1066-6T->G homozygote as well as from unrelated controls, including one obligate noncarrier (HA169) from an A-T family. Cell pellets were lysed essentially as described previously (10), and protein concentrations were determined by the Bradford method (Bio-Rad). Forty μg of total protein per lane were separated on 4.2% SDS-polyacrylamide gels, followed by blotting onto Hybond C-extra nitrocellulose membrane (Amersham) and overnight incubation with a 1:1000 dilution of monoclonal antibody 2C1 raised against the COOH-terminal portion of ATM (amino acids 2577–3056; GeneTex; Ref. 42). After incubation with secondary antibody, signals were detected by enhanced chemiluminescence (Amersham) followed by autoradiography. The same blot was subsequently probed with a 1:500 dilution of the monoclonal antibody Ab-2 raised against the catalytic subunit of DNA-dependent protein kinase (Oncogene Research), which served as the internal control for loading and integrity of total protein.

Clinical data were evaluated retrospectively from all available patient records. Patients were grouped by their ATM genotype, and the characteristics of patients and tumors were compared by χ² tests. Results were considered to be significant for P < 0.05, or where multiple testing with six independent mutation genotypes was performed, for P < 0.008 following Bonferroni’s correction. CIs (95% or 99%) of ORs were calculated by χ²-based approximation using the Win Episcope 1.0 software package.

RESULTS

Mutation Scanning of the ATM Gene. We performed a mutation analysis of genomic PCR products amplified from DNA samples of 192 consecutive breast cancer patients by SSCP analysis of all coding exons and flanking intron sequences of the ATM gene. Subsequent direct sequencing of PCR products with an aberrant migration identified a total of 21 different sequence alterations within the ATM coding region (Table 1). One of the two truncating mutations was a frameshift deletion, 3801delG in exon 28 of the ATM gene (Fig. 1), which was detected in a single patient with bilateral breast cancer. This woman had been diagnosed by age 50 for her first breast cancer and had received postoperative local regional radiation therapy after mastectomy. By age 69, she had developed a second primary of the contralateral breast, which was irradiated after breast-conservative surgery. She died from cancer by the age of 74 years.

An additional 16 exonic alterations were amino acid substitutions dispersed throughout the whole coding region (Table 1), classifying amino acid substitutions as the major type of sequence alteration within the ATM open reading frame. Homozygotes were identified for the amino acid substitution D1853N, and compound heterozygosity was found in five patients that also involved the frequent D1853N polymorphism. One patient was a compound heterozygote for the D1853N and D1853V substitutions, and two patients carried the

Table 1 Mutations of the ATM gene in 192 breast cancer patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nucleotide</th>
<th>Location</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frameshift mutation</td>
<td>3801delG</td>
<td>Deletion of G at 3801–3802</td>
<td>Exon 28</td>
</tr>
<tr>
<td>Splicing mutation</td>
<td>1066-6T-&gt;G</td>
<td>T-&gt;G at 1066-6</td>
<td>Intron 10</td>
</tr>
<tr>
<td>Amino acid substitutions</td>
<td>S49C</td>
<td>C-&gt;G at 146</td>
<td>Exon 5</td>
</tr>
<tr>
<td></td>
<td>I550V</td>
<td>A-&gt;G at 1648</td>
<td>Exon 13</td>
</tr>
<tr>
<td></td>
<td>P604S</td>
<td>C-&gt;T at 1810</td>
<td>Exon 14</td>
</tr>
<tr>
<td></td>
<td>S707P</td>
<td>T-&gt;C at 2119</td>
<td>Exon 15</td>
</tr>
<tr>
<td></td>
<td>F858L</td>
<td>T-&gt;G at 2572</td>
<td>Exon 19</td>
</tr>
<tr>
<td></td>
<td>I1024R</td>
<td>C-&gt;G at 3161</td>
<td>Exon 24</td>
</tr>
<tr>
<td></td>
<td>L1420F</td>
<td>T-&gt;C at 4258</td>
<td>Exon 31</td>
</tr>
<tr>
<td></td>
<td>V1570A</td>
<td>T-&gt;C at 4709</td>
<td>Exon 33</td>
</tr>
<tr>
<td></td>
<td>S1691K</td>
<td>A-&gt;C at 5071</td>
<td>Exon 36</td>
</tr>
<tr>
<td></td>
<td>D1853N</td>
<td>G-&gt;A at 5557</td>
<td>Exon 39</td>
</tr>
<tr>
<td></td>
<td>D1853V</td>
<td>A-&gt;T at 5558</td>
<td>Exon 39</td>
</tr>
<tr>
<td></td>
<td>A2274T</td>
<td>G-&gt;A at 6820</td>
<td>Exon 49</td>
</tr>
<tr>
<td></td>
<td>G2287A</td>
<td>G-&gt;C at 6860</td>
<td>Exon 49</td>
</tr>
<tr>
<td></td>
<td>C2464R</td>
<td>C-&gt;T at 7390</td>
<td>Exon 52</td>
</tr>
<tr>
<td></td>
<td>S2592C</td>
<td>C-&gt;G at 7775</td>
<td>Exon 54</td>
</tr>
<tr>
<td></td>
<td>G2772R</td>
<td>G-&gt;A at 8314</td>
<td>Exon 59</td>
</tr>
<tr>
<td>Synonymous substitutions</td>
<td>735C/T</td>
<td>C-&gt;T at 735</td>
<td>Exon 9</td>
</tr>
<tr>
<td></td>
<td>1020C/A</td>
<td>A-&gt;T at 1020</td>
<td>Exon 10</td>
</tr>
<tr>
<td></td>
<td>2193C/T</td>
<td>T-&gt;C at 2193</td>
<td>Exon 16</td>
</tr>
<tr>
<td></td>
<td>1576C/T</td>
<td>C-&gt;T at 4578</td>
<td>Exon 32</td>
</tr>
</tbody>
</table>

* Het, heterozygote; Hom, homozygote. F858L is linked with P1054R on the same allele.
S707P substitution apparently in trans with D1853N. The A2274T and the G2772R substitutions were similarly found in patients who were heterozygous for the D1853N polymorphism, but the phase could not be deduced in these single cases.

An additional four exonic alterations were synonymous sequence variants (Table 1). Because these do not change the coding sequence, they were considered neutral variants, although the 735C→T substitution may be able to enhance the alternative splicing of ATM exon 9 (43). In addition, several intronic alterations were identified, including the common polypyrimidine tract mutation 1066-6T→G, which could be shown to cause aberrant splicing that results in a premature stop codon (underlined). Altogether, 89 (46%) of the breast cancer patients in our initial study cohort were carriers of at least one sequence change, whereas the other five were alive, and late radiation-related toxicities did not exceed grade II according to LENT-SOMA score criteria.

Characterization of the Splicing Mutation 1066-6T→G. The most frequent truncating A-T mutation in our sample of breast cancer patients was a splicing mutation, polypyrimidine tract substitution 1066-6T→G within the acceptor splice site of intron 10 of the ATM gene. Two patients in our core group of 192 breast cancer cases were heterozygous for the intronic transversion, but when we extended our screening to the whole cohort, using an Rsal restriction enzyme based assay, five additional breast cancer cases were identified, raising the total number to 7 in 1000 (0.7%). In addition, this mutation was observed in 3 of 500 (0.6%) random individuals with unknown phenotype from the general population of Lower Saxony.

We had initially identified the 1066-6T→G mutation in the homozgyous state as the disease-causing mutation in a German A-T patient of Turkish descent (Fig. 2). The 10-year-old boy developed ataxia at 2 years of age and now presents all typical symptoms of classical A-T, including telangiectasia, IgA deficiency, chromosomal instability, elevated AFP concentration, and the recent occurrence of non-Hodgkin lymphoma. To confirm the pathogenic effect of the subtle splice site alteration, we established a lymphoblastoid cell line from this patient and assessed the residual protein and RNA levels of ATM expression. Only traces of full-length ATM protein could be detected in the patient’s cell line by Western blot analyses using an antibody against the COOH-terminal portion of ATM (Fig. 2). To determine the underlying mechanism more closely, we performed a quantitative analysis of ATM mRNA splicing. Repeated reverse transcription-PCR analyses confirmed the loss of exon 11 in 93 ± 4% of ATM mRNA transcripts in this cell line (Fig. 3). A parallel analysis of several cell lines and tissues obtained from individuals without this mutation showed that the alternative splicing of exon 11 usually occurs in 5–15% in normal cells (Fig. 3).

When we examined the ATM transcripts in primary lymphocytes isolated from three unrelated breast cancer patients of our study who were heterozygous for the mutation, the proportions of exon 11 skipping were 40–60% (Fig. 3). Thus, the 1066-6T→G mutation is a common A-T mutation in German breast cancer patients, and ATM inactivation in these patients occurs in the range expected for A-T heterozygotes. The clinical characteristics of breast cancer in all seven identified heterozygotes are compiled in Table 2. Six patients received postoperative radiotherapy without severe acute complications. After a follow-up of 3 years, two of the patients had died from cancer, whereas the other five were alive, and late radiation-related toxicities did not exceed grade II according to LENT-SOMA score criteria.

Frequency and Clinical Relevance of Other Common ATM Gene Alterations. Apart from the 1066-6T→G mutation, the more common ATM gene mutations in our breast cancer cohort were of the missense type. To gain more insight into whether some of these changes predispose to breast cancer, we screened our total group of 1000 unselected breast cancer patients and a cohort of 500 random blood donors from the general population of Lower Saxony for the presence of the more frequent ATM substitutions (Table 3). No difference was observed in the frequency distribution of the common polymorphism D1853N between cases and random individuals (allele frequency, 0.13). However, the rarer missense substitutions with a carrier frequency >1% were, as a group, more prevalent in the breast cancer cohort than in the comparison group (7.9% versus 5.3% of alleles; P < 0.01). The OR of the cumulative frequencies of these amino acid substitutions between cases and random individuals was 1.6 (99% CI, 1.1–2.6; Table 3). This difference remained significant after we excluded the hypothesis-generating cohort of the first 192 patients. The most prominent trend was observed for the S707P substitution in exon 15 of ATM, which had a >2-fold increased prevalence in the breast cancer cohort compared with random blood donors (OR = 2.4; 95% CI, 1.0–5.8; Table 3). One of the patients had a sister who also was affected by breast cancer, and she also had inherited the S707P allele. Tendencies were less pronounced for the missense substitutions L1420F, P1054R, and F858L, and similar to the D1853N polymorphism, no trend was observed in case of the D1853N substitution (Table 3).

Although their frequencies as well as the identification of homozy-
Fig. 2. Identification of splicing mutation 1066-6T→G in a homozygous A-T patient. Left panel, direct genomic sequencing of exon 11 and the flanking sequence of intron 10. Sequencing of the antisense strand is shown. The sequence on the left is from a A-T patient homozygous for the 1066-6T→G mutation. The location of the substitution within the polypyrimidine tract of intron 10 is written below (+). The mutation creates a RsaI recognition sequence (underlined). Right panel, Western blot analysis of lymphoblastoid cell extracts from an obligate non-A-T carrier (left lane) and the homozygous A-T patient (right lane). DNA-PKcs (470 kDa; top band) served as internal loading and quality control. Only traces of ATM protein (370 kDa; bottom band) were detectable in the patient compared with the control.

Fig. 3. Exon skipping in carriers of the ATM splicing mutation 1066-6T→G. Relative peak areas correspond to the relative amounts of product with and without ATM exon 11. One representative experiment is shown for each investigated sample. Top left, homozygous A-T patient [exon 11(+), 7%; exon 11(−) 93%]; middle left, noncarrier control [exon 11(+), 88%; exon 11(−), 12%]; bottom left, control reaction without RNA; top right, breast cancer patient 1, heterozygous carrier [exon 11(+), 60%; exon 11(−), 40%]; middle right, breast cancer patient 2, heterozygous carrier [exon 11(+), 53%; exon 11(−), 47%]; bottom right, breast cancer patient 3, heterozygous carrier [exon 11(+), 44%; exon 11(−), 56%].

DISCUSSION

We investigated the whole ATM coding sequence and flanking untranslated regions in a hospital-based sample of 192 consecutive breast cancer patients; we also screened the most common ATM gene alterations in a total of 1000 breast cancer patients and 500 controls. In contrast to previous studies, our sample was, to our best knowledge, not selected by age, family history, or radiation-related adverse effects and therefore did not preclude the characterization of low-penetrance mutations. We used genomic DNA for mutational screening to avoid leaving open the possibility that these ATM variants, although not classical A-T mutations, could modulate the course and prognosis of breast carcinoma. In a comparison of clinical characteristics of patients carrying the S707P substitution with those of noncarriers, we failed to observe differences regarding the age at onset, but we found a markedly higher proportion of axillary node-positive patients (17 of 26; P < 0.001) and a higher proportion of bilateral breast cancer (5 of 26; P = 0.02) among the S707P heterozygotes. Altogether, the S707P substitution was five times more frequent in patients with bilateral breast cancer (P < 0.001) than in our random sample from the general population.

5 ATM gene mutation database (http://www.vmresearch.org/atm.htm).
cating that it is a common A-T mutation. The splicing mutation 1066-6T→G was found twice in our initial cohort and represents another frequent A-T mutation as discussed below. With the appropriate adjustment for the 75% detection rate of our screening method, it follows that ~1 in 50 breast cancer patients appears to be heterozygous for a truncating ATM mutation. This value is intermediate between previous studies that had focused on early-onset breast cancer (26, 32) and is consistent with the view that the number of A-T mutations now appears to be the only detected ATM mutation in a patient with a classical course of A-T and with deficiency of ATM protein. (b) It led to extensive skipping of exon 11 in ATM mRNA transcripts from the homozygous A-T patient’s cell line (93%) as well as in lymphocyte samples from three heterozygous breast cancer patients (40–60%). The loss of exon 11 results in a frameshift and premature termination codon, as do the vast majority of A-T mutations, and this is not a prominent alternative splicing event in normal cells (Refs. 37, 46, and this study). (c) Lymphoblastoid cell lines established from our breast cancer patients heterozygous for the 1066-6T→G mutation have been shown to exhibit increased cellular radiosensitivity as assessed by whole chromosome painting and by micronucleus formation tests (47, 48). (d) Cosegregation of the 1066-6T→G mutation with breast cancer has been demonstrated in some breast cancer families. Thus, the 1066-6T→G substitution now appears to be the most common truncating mutation in Northern European A-T heterogeneous. In our small sample, the frequency of 1066-6T→G was not different between patients (0.7%) and random individuals (0.6%), indicating a need for further validation studies with larger sample sizes and with defined age-matched cancer-free individuals as controls. Additional insight can also be gained from screening this mutation in large breast cancer families to determine the age-dependent penetrance and the size of the relative risks for breast cancer and other malignancies that are associated with heterozygosity for this frequent A-T mutation.

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at diagnosis, (years)</th>
<th>Follow-up (months)</th>
<th>Family history</th>
<th>Histology</th>
<th>TNM</th>
<th>Treatment volume/ Total dose</th>
<th>Acute toxicity (CTC)</th>
<th>Late toxicity (LENT-SOMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL</td>
<td>69</td>
<td>18</td>
<td>0</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Breast, 50.4 Gy</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
<tr>
<td>HR</td>
<td>35</td>
<td>19</td>
<td>2</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Breast, 50.4 Gy</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
<tr>
<td>KS</td>
<td>63</td>
<td>13</td>
<td>0</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Breast, 50.4 Gy</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
<tr>
<td>ME</td>
<td>36</td>
<td>24</td>
<td>1</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Refused radiotherapy</td>
<td>Grade II</td>
<td>Grade 0</td>
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<tr>
<td>EL</td>
<td>54</td>
<td>15</td>
<td>0</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Breast, 52.2 Gy</td>
<td>Grade 1</td>
<td>Grade 0</td>
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<tr>
<td>MW</td>
<td>50</td>
<td>34</td>
<td>3</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>Breast, 54 Gy</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
<tr>
<td>ES</td>
<td>62</td>
<td>28</td>
<td>0</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>None</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
<tr>
<td>MW</td>
<td>74</td>
<td>34</td>
<td>3</td>
<td>Invasive ductal</td>
<td>(pT_{1054}pN_{1853}M_{0})</td>
<td>None</td>
<td>Grade 1</td>
<td>Grade 0</td>
</tr>
</tbody>
</table>

* Family history is given as the number of first- and second-degree relatives with breast cancer.

Tumors are characterized according to the TNM classification (68) with one patient (HR) harboring a multifocal carcinoma, and one (ES) suffering from a regional recurrence at 74 years.

None of the A-T heterozygotes showed radiotherapy-related toxicities higher than grade II.

Two patients died from cancer at age *3* 38 years and *3* 56 years.

### Table 3

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Breast cancer (n = 1000)</th>
<th>Controls (n = 500)</th>
<th>(p^p)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S707P</td>
<td>28 heterozygotes (0.03)</td>
<td>6 heterozygotes (0.01)</td>
<td>0.05</td>
<td>2.4 (1.0–5.6)</td>
</tr>
<tr>
<td>F858L*a</td>
<td>35 heterozygotes (0.04)</td>
<td>13 heterozygotes (0.03)</td>
<td>0.35</td>
<td>1.4 (0.7–2.7)</td>
</tr>
<tr>
<td>P1054R</td>
<td>1 heterozygote (&lt;0.01)</td>
<td>24 heterozygotes (0.05)</td>
<td>0.24</td>
<td>1.4 (0.8–2.2)</td>
</tr>
<tr>
<td>L1420F</td>
<td>1 heterozygote (&lt;0.01)</td>
<td>17 heterozygotes (0.03)</td>
<td>0.16</td>
<td>1.5 (0.9–2.7)</td>
</tr>
<tr>
<td>D1853V</td>
<td>12 heterozygotes (0.01)</td>
<td>4 heterozygotes (0.01)</td>
<td>0.97</td>
<td>1.0 (0.3–3.0)</td>
</tr>
<tr>
<td>D1853N</td>
<td>235 heterozygotes (0.24)</td>
<td>74 heterozygotes (0.23)</td>
<td>0.79</td>
<td>1.0 (0.8–1.4)</td>
</tr>
<tr>
<td>12 homozygotes (0.01)</td>
<td>4 homozygotes (0.01)</td>
<td>0.97</td>
<td>1.0 (0.3–3.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Carrier frequencies are given as numbers of heterozygotes or homozygotes, respectively, with the corresponding percentages given in parentheses.

* P was calculated from the comparison of allele frequencies to account for both heterozygotes and homozygotes.

* ORs and 95% CIs are shown for each substitution separately as well as for the whole group of rare missense mutations, excluding the common D1853N polymorphism. Note that the comparison group is not composed of age-matched controls and that the given ratios do not represent the relative risks conferred by each of the substitutions.

* F858L is linked with P1054R-F858L haplotype (40).

* Total number of controls tested for codon 1853 variants was 325.

* P and OR for cumulative analysis of non-D1853N missense substitutions.
The pathogenicities of the 16 identified nontruncating mutations cannot accurately be predicted at the present stage. Any formal test of the association between A-T heterozygosity and breast cancer in a population-based sample is hampered not only by the very large sample sizes required, but also by the large number and variety of missense mutations whose allelic effects on protein function and DNA repair capacity are unknown at present. Although none of the missense substitutions in our study targeted a residue known to be crucial for ATM function, 12 of the 16 substitutions affected residues that are identical in murine atm, the exceptions being I550V, F858L, V1570A, and S1691R (49). Only five of the amino acid substitutions were located in the COOH-terminal third of the protein, where a cluster of missense substitutions had previously been implicated in malignancy (50) and which harbors the conserved phosphatidylinositol 3′-kinase signature motifs and a putative domain shared by members of the FRAP, ATM, and TRRAP subfamilies (8, 51).

Eight of the 16 missense substitutions were found to be polymorphisms, including the D1853N and the P1054R substitutions, which have been proposed as genetic modifiers of cancer penetrance (52, 53). Although the most frequent polymorphism, D1853N, showed the same prevalence in cases and random individuals, we found a significant excess of the less common missense substitutions in our breast cancer patients. A similar observation has been reported by others in a recent study of patients in the United States with early-onset breast cancer (54). The S707P substitution in our study appeared to be slightly more frequent in our breast cancer group than in the comparison cohort. On the other hand, homozygotes for the F858L-P1054R and L1420F substitutions have been identified only among our breast cancer patients thus far, and moderate risks cannot be excluded. Several other missense substitutions seem to be rare or even private changes, which will make it difficult to obtain rapid answers from case-control association studies or from the identification of homozygotes. Quantitative in vitro and in vivo expression analyses are required and have been initiated to further characterize these amino acid substitutions and their potential effects on ATM protein stability and/or function.

None of the patients in our study who were heterozygous for ATM gene alterations had a higher-degree acute or late normal tissue reaction related to radiotherapy, indicating that there is no clinically recognized contraindication for postoperative radiotherapy in A-T heterozygous patients. This finding is consistent with previous reports on A-T heterozygotes with breast cancer (25, 26, 64) and with the absence of truncating ATM mutations in cancer patients selected by severe acute radiation reactions (27–30, 65). It does not exclude, however, that A-T heterozygotes may be more susceptible to the carcinogenic effect of ionizing radiation because of an increased intrinsic cellular radiosensitivity (19, 66). In the present study, the frameshift mutation 3801delG was uncovered in one patient who had developed a contralateral breast cancer almost 20 years after receiving radiotherapy for the first tumor, and the S707P missense substitution also was more frequent in patients with bilateral disease. The occurrence of bilateral breast cancer in A-T heterozygotes has been documented by other authors (25, 32), but even if one mutant ATM allele predisposes affected individuals to the development of a radiation-induced second malignancy, alternative treatment or omission of radiotherapy for cancer in A-T heterozygotes is not recommended and can be counterproductive (64). Further investigations are needed to address the effectiveness of strategies to reduce radiation doses or modify therapy for A-T heterozygotes.

In summary, we have identified and characterized a heterogeneous spectrum of ATM gene alterations in a large hospital-based cohort of unselected breast cancer patients. The identification of frequent mutations in our population will simplify screening and enable prospective studies in a clinical research setting. The results presented here provide a basis for future investigations of the functional and clinical impact of ATM gene variations and the magnitude of the relative cancer risk conferred by each of the several identified substitutions.

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Spectrum of ATM Gene Mutations in a Hospital-based Series of Unselected Breast Cancer Patients

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